THE INFLUENCE OF SELECTED FACTORS ON ERYTHROCYTE SEDIMENTATION RATE IN THE DOG AND CAT

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INTRODUCTION

Although the erythrocyte sedimentation rate (ESR) has been employed routinely as a non-specific diagnostic aid in clinical medicine, the blood factors which are responsible for this phenomenon have remained obscure. Prominent investigators have concluded that so little is understood about the complex factors affecting ESR that laboratory results have occasionally permitted erroneous evaluations (Lipford 1966).

The accurate assessment of a patient provided by laboratory examination of the blood has been desired by practicing veterinarians. The value of the ESR as a diagnostic aid has been questioned and debated, particularly in veterinary medicine (Morgan 1966). The object of this study has been to determine what effect selected plasma factors have in influencing ESR in dogs and cats.

REVIEW OF THE LITERATURE

The phenomenon of erythrocyte sedimentation has been arbitrarily divided into four stages (Fahraeus 1929). He described the first stage as rouleau formation by erythrocytes in the blood plasma. The second stage was described as aggregation or agglomeration of the cells. The third stage was regarded as the settling of these aggregated cells in the plasma. Finally he indicated the last stage was the accumulation or packing of the aggregates in the bottom of the container.

Historical Developments

As early as Galen and Hippocrates, blood was observed to separate into fluid and semi-solid components when removed from the host (Fahraeus 1929). Variations in the rate of development and relative size of these components were observed when the blood of diseased and normal humans was examined. These differences were proposed to be due to an imbalance of the four fluids (humours) of the blood and were the supporting evidence for the humoral theory of disease (Fahraeus 1929). This theory became widely accepted among physicians for many centuries. Adherents of the humoral theory of disease regarded blood-letting as an acceptable therapeutic measure. This practice represented an attempt to re-establish the balance of the four humours and continued into the 19th Century.

Blood from diseased humans differed from normal human blood in that there were variations in the rate of development and size of the buffy coat or crusta inflammatoria (Fahraeus 1929). It was in the study of this component that the earliest mention of erythrocyte aggregation and sedimentation were recorded.

An English physician, William Hewson, suggested in 1772 that it was more likely erythrocyte sedimentation rather than delayed coagulation which contributed to the development of the inflammatory crust (Fahraeus 1929). His report of the phenomenon, even though an incidental observation, was probably one of the earliest recorded accounts in which erythrocyte sedimentation was observed and some significance attached to it.

Fahraeus (1929) in his classic review of ESR indicated that John Hunter should be credited with recognizing the possible clinical implication of the phenomenon and quoted his lecture of 1786:

> In all inflammatory dispositions, whether universal or local, the blood has an increased disposition to separate into its component parts, the red globules become less uniformly diffused, and their attraction to one another becomes stronger, so that the blood when out of the vessels soon becomes cloudy or muddy and dusky in its colour, and when spread over any surface it appears mottled, the red blood attracting itself and forming spots of red.

Observations of differences in erythrocyte aggregation and sedimentation continued to be reported, (Nasse 1836; Jones 1843; Gulliver 1845) however, primary interest was in the development and characteristics of the buffy coat (Fahraeus 1929).

Biernacki in 1894 apparently was the first worker to investigate the phenomenon of ESR (Fahraeus 1929). Seemingly unaware of the observations of Hunter, he correlated the severity of the disease process with the relative increase in erythrocyte sedimentation. He proposed that dying red cells secreted plasma factors which hastened erythrocyte sedimentation, and, as the disease increased in severity, more cells died and erythrocyte sedimentation was accelerated (Fahraeus 1929).

Fahraeus (1918) observed that erythrocytes settled faster in the plasma of pregnant women than they did in that of non-pregnant ones. Within the next

few years Fahraeus conducted extensive investigations attempting to correlate ESR results with clinical observations. The results of the early investigations by Fahraeus and his student Westergren provided the foundation for the evaluation of ESR as a diagnostic aid to clinical medicine (Wintrobe 1941).

Apparatus and Techniques for Determination of ESR

In attempting to improve the significance of many laboratory examinations, modifications of equipment and techniques have evolved. Determination of ESR has been no exception, but, regardless of apparatus employed, each of the methods has been characterized by the settling of erythrocyte aggregates in a column of blood (Morrison 1941).

Fahraeus indicated in his review (1929) that his initial investigations of ESR employed a simple glass tube 200 mm. in length with an internal diameter of 2.5 mm. The tube proposed by Westergren (1926), which is used in many laboratories, was 300 mm. long, however, the lumen diameter remained at 2.5 mm. The sedimentation pipette described by Linzenmeier and Raunert (1924) contained a bulb in the upper portion of the column and resembled a white blood cell diluting pipette. The functional length of this apparatus was 65 mm., and the internal diameter was 5.0 mm. This tube provided the basic design for the Landau (1933) microsedimentation pipette which was the same length but reduced the bore to 1 mm. Cutler(1940) proposed a tube that was 70 mm. in length and 5.0 mm. in bore diameter. The tube considered by Rourke and Ernstene (1930) was 120 mm. in length and had an internal diameter of 4.0 mm. Wintrobe and Landsberg (1935) proposed a sedimentation tube that was also 120 mm. long, however, their investigations suggested that an internal diameter of 2.5 mm. allowed accurate ESR determinations. The tube described by Smith (1936) was a modification of the Cutler design being 70 mm. in length, but the internal

diameter was reduced to 2.5 mm. Bild (1965) suggested that the microhematocrit capillary tube, which has a length of 75 mm. and internal diameter of 1.2 mm., would be useful in determining the rate of erythrocyte sedimentation.

Various techniques have been described in the use of these tubes. Berczeller and Wastl (1923), Lundgren (1927), and Washburn and Meyers (1957) have suggested the use of inclined rather than vertical tubes to determine ESR, but this method has never gained popularity.

The more common practice in utilizing variously modified tubes has been to measure in mm. the fall of the settling erythrocytes which occurred during the first hour. Some investigators proposed different methods of determining ESR values. The results that were obtained utilizing the Rourke-Ernstene tube depended on determining the slope of the period of constant fall of aggregated erythrocytes. ESR results were reported in mm. fall per minute. The method of Cutler (1940), employing his sedimentation tube, determined the graphic curve of erythrocyte sedimentation in one hour. Results were reported in minutes when the Linzenmeier tube was utilized to determine the rate of erythrocyte sedimentation. The time for erythrocytes to descend the first 18 mm. in the blood column was measured. Other proposed sedimentation tubes were read in mm. fall in one hour.

Physical Factors Altering ESR

Several physical and technical factors have been noted to alter the rate of erythrocyte sedimentation. The height of the blood column in the sedimentation tube influenced the amount of settling which occurred during the first hour of observation. The investigations reported by Ham and Curtis (1938) indicated that in tubes of the same internal diameter but of different length, the longer tubes packed more gradually and produced an accelerated ESR. In tubes over 200 mm. in length the differences were not felt to be significant

as packing was not thought to be an altering factor during the first hour.

When the internal diameter of the sedimentation tube was between 3 mm. and 11 mm., this factor did not alter ESR (Ham and Curtis 1938). The minimum internal diameter of the sedimentation tube found not to influence ESR was 2.5 mm., Wintrobe and Landsberg (1935); Nichols (1942); Diggs (1966); Morgan (1966). Some controversy existed about the minimum diameter, however, for Ham and Curtis (1938) reported that decreasing the internal diameter from 3 mm. to 2.5 mm. resulted in a 27% decrease in ESR. It was generally accepted that bore diameter of 2 mm. or less caused extensive slowing of ESR and irregularities in the settling of the erythrocyte column (Ham and Curtis 1938). This observation suggested that the internal circumference of a small tube was disproportionately large in relation to its area of cross section and, therefore, had produced a relatively great frictional surface at the wall of the tube with consequential retarding of sedimentation (Ham and Curtis 1938).

The effect that anticoagulants have on altering ESR was not recorded frequently in the literature. Those anticoagulants, primarily the oxalates and citrates, which reduce erythrocyte volume (Henry 1964) were noted to alter ESR in the review by Morgan (1966). It was suggested that excess anticoagulant accelerated the ESR, particularly heparin and the citrates, Fahraeus (1929); Ham and Curtis (1938); Nichols (1942); Diggs (1966). Improper mixing of anticoagulant and blood also was incriminated in altering the ESR determination, Landau (1933); Ham and Curtis (1938); Nichols (1942).

Closely allied with the misuses of anticoagulants was the time of holding of the blood sample prior to determination of ESR. The anticoagulants which reduced erythrocyte cell volume altered ESR if the blood was retained over 3 hours prior to examinations (Ham and Curtis 1938). Most of the commonly employed anticoagulants were not found to prevent alterations in the ESR if the

blood was stored under refrigeration for 24 hours prior to ESR determination (Ham and Curtis 1938).

The salts of ethylenediametetracetate were not thought to alter the erythrocyte volume (Henry 1964), and were, therefore, not likely to produce decreases in ESR if determinations were performed within a few hours following collection. Melville and Rifkind (1959) investigated the use of an EDTA-citrate mixture and its effects upon ESR. They indicated that their anticoagulant had not altered the ESR and that precise determinations were possible if the blood was retained under refrigeration for 24 hours.

Room temperature has been demonstrated to alter the ESR. High room temperatures were reported to increase the ESR while low room temperatures decreased the rate of erythrocyte sedimentation, Wartman (1946); Manley (1957); Morgan (1966). Manley (1957) noted that blood in the Wintrobe tube was less likely to be affected by changes in room temperature than was that in the Westergren tube. Small variations in room temperature reportedly have not altered the ESR significantly, Ham and Curtis (1938); Wartman (1946).

It was widely accepted that inclination of the sedimentation tube altered ESR. Wintrobe and Landsberg (1935) found that an inclination of the sedimentation tube of only 2.3% increased the settling velocity by 30%. Inclination of the tube facilitated streaming of plasma along the uppermost wall of the sedimentation tube, and the aggregations of erythrocytes met with much less resistance as they descended (Ham and Curtis 1938).

Landau (1933) reported a specific technical factor which significantly altered the ESR determination using his microsedimentation pipette. Mixing of excessive air in the collected sample was instrumental in causing a decreased ESR (Landau 1933). Improper mixing of sodium citrate and blood caused an increased ESR or irregular results (Nichols 1942).

Physiological Factors Influencing ESR

Certain physiological factors were reported to influence the ESR. The explanations for these findings were ill defined. Sedimentation rates were reported to be faster in normal adult women than in normal adult men, Cutler (1940); Nichols (1942). Pregnancy was noted as causing increases in the ESR. This observation was indicated frist by Fahraeus (1918) and was confirmed repeatedly by other workers as noted in the review by Nichols (1942).

Controversy has existed about the effect of the age of the host on ESR. The literature suggested that higher sedimentation rates were anticipated in the elderly and that sedimentation rates normally increased with age, Nichols (1942); Wilhelm and Tellisch (1951). Vigorous exercise and excitment were found to increase the rate of erythrocyte sedimentation (Nichols 1942).

Erythrocytic Factors Affecting ESR

The number of red cells in the blood sample was described initially as a major factor influencing ESR by Fahraeus (1929). Interpretations of the increased ESR observed in anemia indicated that the plasma factors exerted more influence when there were fewer red cells per unit of blood volume (Wintrobe 1941). Erythrocyte rouleau and aggregate formation occurred at a faster rate; aggregates proceeded to descend sooner and met with less resistance from other aggregated red blood cells (Ham and Curtis 1938).

If an anemia was found to be present the clinical implications of an elevated ESR often were debated (Cutler 1938). Investigators stimulated by this predicament sought methods to enhance the value of ESR in the presence of decreased erythrocytes. Rourke and Ernstene (1930), Wintrobe and Landsberg

(1935); Cutler (1938); Wintrobe (1941); and Schalm (1965) have proposed ESR correction charts which compensated for anemia.

The use of these charts has been severely and widely criticized by many workers as indicated in the discussion by Diggs (1966). The major criticism was that the compensatory graphs tended to overcorrect the ESR for anemia and that the interpolation of results exceeded the accuracy of the technique of ESR determination (Diggs 1966).

Many clinical pathologists have been dismayed by this dilemma and have been in poor agreement as to a satisfactory compromise (Morgan 1966). It became, therefore, a common practice to report both the corrected and uncorrected ESR when an anemic blood sample was examined, Lipford (1966); Diggs (1966).

The effect of erythrocyte size on ESR values was held in controversy by many workers. In his review Phear (1957) reported that early workers, Marloff (1919); Barker (1922); and Ohno (1926) investigating ESR in red blood cells suspended in Hayem's fluid concluded that ESR was correlated lineally with an increase in erythrocyte radius and mean corpuscular hemoglobin concentration (MCHC). It should be noted that in these experiments rouleaux was not observed as the cells fell individually. Newham and Martin (1928); Fahraeus (1929); and Phear (1957) concluded from their experimental investigations that the cell size had not influenced roulesu or sedimentation. Ham and Curtis (1938) deducted from experimental studies that macrocytic red blood cells produced elevations in ESR.

Cutler (1938) and Wintrobe (1941) indicated that it was the size of the cell aggregates which determined ESR and that this factor was independent of cell size. Poole and Summers in 1952 compared ESR values when macrocytic erythrocytes were exchanged with normocytic red blood cells (Phear 1957). They concluded that macrocytic red blood cells settled slightly faster in normal

plasma than normal erythrocytes. Phear (1957) found from experimental investigations there was a high degree of positive correlation between red blood cell size and ESR, and that there was no correlation between ESR and MCHC. Miale (1962) supported this observation, and stated that in microcytic anemia the corrected ESR was overcorrected due to the small erythrocyte. Diggs (1966) relegated erythrocyte size to an insignificant role in affecting ESR, but failed to cite experimental evidence which supported this conclusion.

The shape of the erythrocyte was found to inhibit rouleau formation and aggregation, and this factor produced a decreased ESR (Phear 1957). This was confirmed in observations of blood from humans afflicted with sickle cell disease or conditions where spherocytes were numerous, Phear (1957); Miale (1962); Diggs (1966) without citing experimental evidence attributed minor significance to the shape of erythrocytes, but further stated that correcting ESR for anemias characterized by marked poikilocytosis was misleading and encouraged erroneous evaluations.

The effect of hemoglobin content of the erythrocyte on ESR has been a cause for concern on the part of several workers. It was observed that ESR was independent of hemoglobin concentration, Nichols (1942); Phear (1957).

It was accepted generally that the specific gravity of the red cell did not alter appreciably the ESR even when compared with the relative specific gravity of the plasma, Nichols (1942); Phear (1957). It was accepted also that the use of capillary, arterial or venous blood did not alter the ESR value as similar results were obtained regardless of source, Landau (1933); Wintrobe (1933); Smith (1936); Nichols (1942).

Plasma Factors Affecting ESR

The influence of pH on ESR was documented in the literature (Nichols 1942).

Although evidence was not conclusive, investigations tended to support the contention that acidosis retarded ESR and alkalosis accelerated it (Nichols 1942). Increased oxygenation or carbon dioxide retention played a questionable role in altering the ESR (Nichols 1942).

It was suggested that plasma viscosity influenced ESR. However, experimental evidence failed to correlate changes in viscosity with changes observed in ESR, Nichols (1942); Phear (1957). Plasma specific gravity was not incriminated as a major factor influencing ESR, Cutler et al. (1938); Nichols (1942); Phear (1957).

Haurowitz (1961) stated that normal human plasma contains 6.5-7.0 gm. of protein and 1 gm. non-protein material per 100 ml. He indicated it was the protein content of plasma which was the significant factor affecting specific gravity, refractive index and other physical characteristics which, in turn, influence ESR. This statement required that the non-protein content of plasma be regarded as constant which was often not the case. However, he was able to conclude that changes in the protein content, particularly the relative per cent contribution to the plasma of its fractions, significantly influenced the ESR.

Wuhrman and Wunderly (1960) devised an experiment which demonstrated the non-specificity of the erythrocyte sedimentation. Washed erythrocytes were placed in solutions of glycogen and pectin and observed to determine the ESR. The results indicated that any desired ESR could be produced by varying the concentration of pectin in the protein-free solution. Pectin, a polysaccharide of high molecular weight, was shown to be an asymmetrical molecule of relatively high molecular weight Wuhrman and Wunderly (1960). Meyer et al. (1945) concluded from *in vivo* and *in vitro* investigations that highly asymmetrical molecules of high molecular weight caused the ESR to be increased. The non-

specificity of factors influencing ESR was indicated also by these investigators.

According to Fahraeus (1929), Hewson in the late 19th Century observed that red blood cells settled slower in defibrinated blood than whole blood. Recalling that the primary interest of investigators of this time was the development of the buffy coat, it was understandable that little significance was attributed to this observation. Fahraeus (1918) in his studies of ESR on the plasma of pregnant women incriminated increased fibrinogen levels as the factor responsible for acceleration of ESR. Numerous other studies both clinical and experimental, supported the supposition that increased fibrinogen increases the ESR, Cutler (1932); Gilligan and Ernstene (1934); Wintrobe and Landsberg (1935); Ham and Curtis (1938); Ropes et al. (1939); Shedlovsky and Scudder (1942); Gray and Mitchell (1942); Nichols (1942); Meyers et al. (1953); Wuhrman and Wunderly (1960); Miale (1962); Lipford (1966); Diggs (1966); Morgan (1966). Experimental studies indicated that only very slight increases in fibrinogen cause marked acceleration in ESR. Gray and Mitchell (1942) reported the addition of 0.2 gram per cent fibrinogen to whole blood increased the hourly fall from 6 mm. to 24 mm.

The effect of ESR on the other globulins, alpha, beta and gamma, was obscure, with controversy existing on the interpretation of experimental findings, Lucia et al. (1935); Ham and Curtis (1938); Ropes et al. (1939); Shedlovsky and Scudder (1941); Gray and Mitchell (1942); Nichols (1942); Meyers et al. (1953); Wuhrman and Wunderly (1960). Most experimental evidence indicated that gamma globulins were the least effective of the globulins in accelerating the ESR, Ropes et al. (1939); Gray and Mitchell (1942); Meyers et al. (1953). The alpha globulins as a group were incriminated as being capable of accelerating ESR, Ropes et al. (1939); Gray and Mitchell (1942); Meyers et al. (1953). Meyers et al. (1953) suggested that an increase in the alpha 2 fraction was capable of

accelerating ESR. This fraction was felt to contain the plasma factors associated with acute injury and increases coincided with elevations in fibrinogen levels, Wuhrman and Wunderly (1960).

Beta globulins also increased ESR. However, Meyers et al. (1953) reported statistical evidence which indicated that beta and alpha 1 globulins exerted little effect on ESR, their influence being only slightly greater than that of gamma globulin.

Numerous reports on the effects of elevated albumin levels on ESR were found in the literature. Elevations in albumin either caused no change or a decrease in ESR, Ropes et al. (1939); Gray and Mitchell (1942); Nichols (1942); Meyers et al (1953).

The various non-protein factors found in plasma which affect ESR have not been studied extensively. However, recognition that elevation in total plasma cholesterol levels may increase ESR was indicated in several reports, Nichols (1942); McAlpine (1955); Meyers et al. (1953). The lecithins decreased ESR if present in elevated quantities (Nichols 1942).

Reported investigations of the effects of commonly used therapeutic agents were found infrequently in the literature. The use of sulfonamides was reported to decrease ESR, Ropes et al. (1939); Harkness (1950). The use of adrenocorticotropic hormone or the corticosteriods was noted to decrease ESR (Vaughn et al. 1951).

MATERIALS AND METHODS

Collection of Blood Samples

Blood for hematologic examination was collected from dogs and cats from three sources. The most commonly used source was Dykstra Veterinary Hospital at Kansas State University. These animals were primarily in-clinic patients. After initiating the collection of blood from normal cats, it became increasingly apparent that other sources were needed. Therefore, blood was collected from normal cats which were in the possession of the Veterinary Physiology Department and the author's wife. These latter sources provided samples from 24 different normal cats and were distinguished from hospital cases in that they were not identified by clinic numbers.

Blood was collected utilizing aseptic technique. A sample size of four ml. was determined to be the minimum amount required for the desired studies. A 10% solution of the di-sodium salt of ethylenediaminetetraacetate (EDTA) was employed as the anticoagulant at the rate of one drop per five ml. of blood. Care was taken to utilize the minimum required amount of anticoagulant. The cephalic vein was the site of the venipuncture for most of the samples obtained from dogs; whereas, the femoral vein was more commonly utilized for blood collection from cats. The one inch needles used for collection from dogs ranged from 20 to 23 gauge. The needle size which allowed acceptable samples to be collected from cats was 22 gauge, three-quarter inch.

After the collection of blood samples, immediate mixing with the anticoagulant was performed. Any sample which developed indications of coagulation was discarded and another sample collected.

Routine Hematological Examination

Immediately following collection the desired hematological examinations were performed. Packed cell volumes were determined using a microhematocrit centrifuge[1]. Hemoglobin values were determined by spectrophotometric analysis[2] of 0.02 ml. of blood to which had been added six ml. of cyanmethemoglobin reagent[3]. The wavelength setting of the spectrophotometer for this examination was 540 millimicrons. Leukocyte and erythrocyte counts were obtained electronically[4] utilizing the standard techniques for cell enumeration. Blood smears were prepared, air dried and stained with Wright's stain. Differential leukocyte counts were performed by both the author and a registered medical technologist, and, when a total difference of over four per cent was found, the discrepancy was resolved by further examination.

Method of Determining Erythrocyte Sedimentation Rates

All sedimentation rates were determined within four hours of collection. Room temperature was recorded daily and did not vary over six degrees farenheit over the period of study.

Two methods of determining erythrocyte sedimentation were employed on all blood samples and the results compared. The Wintrobe disposable sedimentation tubes and ten unit rack with spirit level and leveling screws[5] were utilized in one method. These tubes were approximately 110 mm. long and had an internal

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[1]International Equipment Co. Boston, Massachusetts.

[2]Coleman Jr. Spectrophotometer, Coleman Instrument Inc., Maywood, Illinois.

[3]Hycel Inc., Houston, Texas.

[4]Coulter Counter Model A, Coulter Electronic, Hialiah, Florida.

[5]Scientific Products, Division of American Hospital Supply Corp., Evanston, Illinois.

diameter of approximately 3 mm.; they have a capacity of approximately 0.6 ml. The sedimentation rate was determined at fifteen minute intervals for one hour.

Sedimentation rate was also determined employing the Landau-Adams micro-sedimentation pipette[1]. This apparatus, which resembled a white cell diluting pipette, was 120 mm. in length, and had a scale ranging from 0 mm. to 50 mm. in 1 mm. divisions. The internal diameter of this pipette was approximately 1 mm. and the functional capacity was approximately 0.1 ml. This tube was a slight modification of the Linsemeier tube and was suggested as a possible means of determining significant erythrocyte sedimentation where only small samples of blood were available (Landau 1933). Veterinarians interested in the health of small domestic animals have indicated the desireability of such a method of ESR determination, Bild (1965). The technique varied from that which the designer proposed in two respects. Landau utilized a five per cent sodium citrate solution in a ratio of one part anticoagulant to four parts whole blood. This solution was drawn into a bulb in the column above the graduations and was thoroughly mixed before being allowed to return to the scaled portion. The blood examined in the present study, however contained EDTA as the anticoagulant and was not drawn into the bulb of the pipette as mixing had been previously effected. The pipette was filled to the 10 mm. graduation mark and then placed in a vertical holder designed for the pipette. For the most part blood sample sedimentation rates were observed simultaneously in the Wintrobe tube and Landau micro-sedimentation pipette. At the end of the observation period the blood was removed from the sedimentation tubes and examined for evidence of coagulation.

Following the completion of these studies the plasma was collected and

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[1] No. A 2472 Clay Adams Inc., New York.

stored at 0° C awaiting subsequent examination.

Determination of the Plasma Total Protein and Plasma Total Cholesterol

When all required plasma samples were available, they were thawed and further studies were performed. Total cholesterol was determined spectrophometrically at 625 mu. employing the Liebermann-Burchardt reagent*.

Total plasma proteins were determined by two methods. One method utilized specific gravity[1] measurements while the second method employed the principle of refractive index[2] in calculation of protein content. The values for each sample were averaged, this value was used in the determination of absolute protein distribution.

Method of Electrophorectic Separation

Electrophoretic analysis of plasma proteins was performed on cellulose polyacetate strips[3]. Duplicate samples were electrophoresed for one hour at 255 volts on different electrophoresis chambers[4]. The barbital sodium buffer had the ionic strength of 0.05 M and a pH of 8.6[5]. Ponceau S[6] was used to stain the electrophoresed proteins. The stained stripe were densimetrically analyzed and the relative percent of each plasma protein was determined[7]. Results

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*Hycel Inc. Houston, Texas.

[1]Banco Density Gradient. Anderson Laboratories. Fort Worth, Texas.

[2]Serum Protometer. Bausch and Lomb. New York.

[3]Sepraphore III Cellulose Polyacetate Strips. Gelman Instruments Co., Ann Arbor, Michigan.

[4]Rapid Electrophoresis Chamber Number 51101. Gelman Instruments Co., Ann Arbor, Michigan.

[5]Buffer Salt Type B. Harleco-Hartman-Leddon Co., Philadelphia, Penn.

[6]Ponceau S Stain, Harleco-Hartman-Leddon Co., Philadelphia, Penn.

[7]Beckman Spinco Analytrol Model B, Spinco Division, Beckman Instruments Inc., Belmont, California.

obtained on the duplicate separations were averaged and absolute values, based on total protein determination, were calculated for albumin, alpha-globulin, beta-globulin, gamma-globulin and fibrinogen.

Division of Blood Data into Classes and Groups

The accumulated data from the animals were placed categorically into three major classes: clinically normal dogs, clinically normal cats, and clinically diseased dogs. The clinical evaluation of each animal was performed by the small animal clinical staff of Dykstra Veterinary Hospital. Dogs were considered to be normal if they appeared healthy and free from obvious disease, if temperature were within normal limits, and if hematological findings were within normal limits as described by Bruner and Wakerlin (1937); Schalm (1965). In addition, their erythrocyte sedimentation rates using the Wintrobe tube were within normal limits proposed by, Simms (1940); Knowles (1955); Bild (1956); and Schalm (1965). Cats were considered similarly except for one major difference. As values for erythrocyte sedimentation for the cat were sparse in the literature, it was not advisable to rely extensively on previously published information as a criterion of normalcy. Therefore, cats with highly elevated ESR appear within the group of normal cats. Dogs with clinical signs of disease were randomly sampled during the period of study. Dogs were considered diseased if the ESR results using the Wintrobe tube exceeded those proposed as normal by Schalm (1965).

Blood was collected only if the size of the dog permitted easy collection. Dogs which had received a therapeutic regimen including antimicrobial or anti-inflammatory drugs were not used as blood donors.

Data from these groups were assembled according to an arbitrary division based on the age of the animal. When the age was not known for certain, it was estimated by at least two members of the clinical staff.

RESULTS AND DISCUSSION

Normal Dogs

Group I, One - Two Months of Age

Routine Hematological Examination

Eight of the ten pups in this group were littermates, and trends in normal values were recognized. Examination of the data (Table 1) obtained from the analysis of hemoglobin concentration, packed cell volume, total red cell counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) suggested that pups in this age group have a macrocytic, normochromic red cell. This observation was supported in the literature (Schalm 1965).

The normal trends established from the examination of percentage and absolute distribution of leukocytes were not defined clearly (Appendix table 1). Examination of the data indicated that the total white cell count of this group was slightly higher than that reported on a comparable age group by Schalm (1965). This result was due to elevations in absolute numbers of lymphocytic cells. The remainder of the absolute distribution of the leukocytes was in close agreement with those numbers reported by Schalm (1965). Schalm indicated, however, that lymphocytes may be relatively high (over 30%) in young pups and that this number will decrease with age. It was observed that the increased number of total leukocytes found in this study was due to increased lymphocytes.

Table 1. Results of Erythrocyte Studies and Blood Chemistries on Normal Dogs One to Two Months Old (Group I)

Animal Hospital Number	Hb[1]	PCV in %	Total RBC x 10^6	MCV[2]	MCH[3]	MCHC[4]	Uncorrected ESR in mm. Wintrobe Minutes 15	30	45	60	Landau-Adams Minutes 15	30	45	60	A/G	Plasma Sp. Gr.	Plasma[5] Cholesterol
66-10874	11.6	36.	5.185	69	22	32.2	0	0	0	0	0	0	0	0	1.64	1.0215	152
66-11582	10.1	31.	3.460	83	27	32.6	0	0	0	0	0	0	0	0.5	2.58	1.0200	210
66-11583	10.0	30.	3.970	69	23	33.3	0	0	0	0	0	0	1.	1.	2.64	1.0185	170
66-11584	9.0	28.	3.290	79	25	32.1	0	0	0	0.5	0	0	0	1.	1.70	1.0190	154
66-11585	9.8	30.	3.600	77	25	32.7	0	0.5	1.	2.	0	0	0	0.5	2.19	1.0190	190
66-11586	10.8	34.	3.830	81	26	31.8	0	0	0	0	0	0	0	0	1.11	1.0200	211
66-11587	10.1	31.	3.150	92	30	32.6	0	0	0	0	0	0	0	0	1.28	1.0200	158
66-11588	9.2	29.	3.330	82	26	31.7	0	0	0	0	0	0	0.5	1.0	2.15	1.0190	134
66-11589	10.1	30.	3.700	75	25	33.7	0	0	0	1.	0	0	0	0	2.18	1.0190	177
66-14140	9.2	31.	3.851	74	22	26.7	0	0	0	0	0	0	0	0	1.35	1.0215	177
Mean	10.0	31.0	3.735	78.1	25.1	31.9									1.88	1.0199	173.0

1. Hemoglobin concentration in gms. per 100 ml. blood.
2. Mean corpuscular volume in cubic microns.
3. Mean corpuscular hemoglobin in micromicrograms.
4. Mean corpuscular hemoglobin concentration in percent.
5. Total cholesterol in mg. per 100 ml. plasma.

Table 2. Total Plasma Protein and Electraphoretogram Values of Normal Dogs One to Two Months Old (Group I)

Animal Hospital Number	Percentage Protein Distribution per 100 ml. Plasma					Absolute Protein Distribution in gms per 100 ml. Plasma					Total Plasma Protein in gms. per 100 ml. Plasma	
	Albumin	Alpha Glob.	Beta Glob.	Fibrinogen	Gamma Glob.	Albumin	Alpha Glob.	Beta Glob.	Fibrinogen	Gamma Glob.	Banco	Protometer
66-10874	55	17	11	11	6	2.84	0.84	0.56	0.54	0.33	5.3	4.9
66-11582	62	14	8	14	2	2.79	0.63	0.36	0.63	0.09	4.5	4.4
66-11583	63	14	9	12	2	2.56	0.54	0.36	0.47	0.07	4.2	3.9
66-11584	54	20	10	13	3	2.26	0.81	0.40	0.51	0.11	4.1	4.1
66-11585	61	15	11	11	2	2.56	0.63	0.47	0.47	0.06	4.3	4.1
66-11586	45	21	13	16	5	1.90	0.95	0.56	0.70	0.21	4.7	4.4
66-11587	50	23	12	12	3	2.21	1.04	0.56	0.55	0.14	4.3	4.7
66-11588	59	16	10	12	3	2.48	0.67	0.39	0.49	0.11	4.2	4.1
66-11589	59	15	11	13	2	2.44	0.62	0.43	0.53	1.08	4.2	4.1
66-14140	51	19	12	12	6	2.54	0.95	0.63	0.58	0.32	5.0	4.9
Mean	56	17.3	10.7	12.4	3.3							4.4[2]
S.D.[1]	5.2	3.2	1.5	1.5	1.8							

1. Standard deviation.

2. Mean of averaged total protein values.

ESR and Plasma Cholesterol

The data accumulated from the ESR determinations (Table 1) suggested that normal dogs in this age group have negligible settling of red blood cells in one hour. Results were uniformly consistent when determined by either technique, the Wintrobe or Landau-Adams sedimentation tubes. The values obtained were well within the limits of ESR proposed in Schalm's correction chart for anemia (Schalm 1965) even though uncorrected ESR were reported in the data.

Determination of plasma cholesterol values (Table 1) suggested a high state of thyroid activity which was anticipated in young pups. All values were below those considered to be normal by Schalm (1965).

Total Proteins, Specific Gravity, Albumin-Globulin Ratio and Electrophoretic Separation.

As was expected from the relatively low specific gravity, plasma protein values were also low (Table 2). These results were in agreement with those reported by Tomada (1963) who reported that one and two month old puppies had total serum protein levels near 4.0 gms./100 ml. Unfortunately, his method of serum protein determination was not described in the English abstract.

The literature did not contain reports of plasma protein separations obtained by electrophoresis on cellulose polyacetate strips on dogs of this age group. Therefore, little information was available with which to compare the results obtained in this study. The electrophoretic separations obtained in the present study generally permitted excellent differentiation of plasma protein components.

In this present group of pups, as was consistently found in the majority

of normal dogs, differentiation of the alpha globulins into distinct subcomponents was obscure. Therefore, division of the alpha globulins was not attempted. The migration of fibrinogen usually was associated with that of the beta globulins and gamma globulins. The absolute fibrinogen values found in this study were similar to those reported in the literature; from the analysis of the electrophoretograms in this study it would appear likely that all of the values reported for fibrinogen were influenced by beta and gamma globulin.

The low total protein values obtained from the study of this age group were anticipated. Low fractions of gamma globulin, albumin, beta globulin, and alpha globulin were found. The relative distribution of protein fractions in the pups of Group I differed from that found in the older dogs of Groups II and III; this was noted particularly in the case of gamma globulin which were low in the pups of Group I.

Normal Dogs

Group II, Six Months - One Year of Age

Routine Hematological Examination

Examination of the data obtained from the determinations (Table 3) for hemoglobin concentration, packed cell volume, total red blood cell counts, MCV, MCH, MCHC agreed with respective values reported in the literature (Schalm 1965) for normal dogs.

Total leukocyte counts and relative and absolute distributions (Appendix Table 2) were in close agreement with those reported for young normal dogs Schalm (1965).

Table 3. Results of Erythrocyte Studies and Blood Chemistries on Normal Dogs Six Months to One Year Old (Group II)

Animal Hospital Number	Hb[1]	PCV in %	Total RBC x 10^6	MCV[2]	MCH[3]	MCHC[4]	Uncorrected ESR in mm. Wintrobe Minutes 15	30	45	60	Landau-Adams Minutes 15	30	45	60	A/G	Plasma Sp. Gr.	Plasma[5] Cholesterol
66- 8799	15.9	47.	5.460	76	26	33.8	0	0	0	0	0	0	0.5	0.5	2.06	1.0265	225
66- 8848	17.0	50.	5.930	74	25	34.0	0	0	0	0	0	0	0.2	0.2	2.16	1.0265	340
66-10844	14.4	44.	4.910	80	26	32.7	0	0	0.5	0.5	0	0	0.7	0.7	1.64	1.0240	137
66-11269	13.0	39.	4.840	72	24	33.3	0	2.	2.	4.	0	0	0	2.	1.42	1.0250	232
66-12207	16.2	48.	5.195	82	28	33.8	0	0	0	0	0	0	0	0	1.50	1.0250	111
66-12557	15.7	49.	5.395	80	26	32.0	0	0	0	0	0	0	0	0	1.98	1.0240	217
66-12780	17.6	52.	6.070	86	29	33.8	0	0	0	0	0	0	0	0	2.0	1.0265	196
66-13145	15.2	47.	5.671	73	24	32.3	0	0	0	0	0	0	0	0.5	1.50	1.0265	184
66-13410	15.7	49.	5.492	79	25	32.0	0	0	0	0	0	0	0	0	1.60	1.0265	254
66-13411	16.6	45.	5.679	69	25	36.9	0	0	0	0	0	0	0	0.5	2.56	1.0240	287
66-13418	18.6	49.	6.545	65	24	38.0	0	0	0	0	0	0	0	0	1.92	1.0265	158
66-14151	14.0	45.	5.430	73	23	31.1	0	0	0	0	0	0	0	0	1.59	1.0235	258
Mean	15.8	47.0	5.551	75.8	25.4	33.6									1.83	1.0254	216.6

1. Hemoglobin concentration in gms. per 100 ml. blood.
2. Mean corpuscular volume in cubic microns.
3. Mean corpuscular hemoglobin in micromicrograms.
4. Mean corpuscular hemoglobin concentration in percent.
5. Total cholesterol in mg. per 100 ml. plasma.

Table 4. Total Plasma Protein and Electrophoretogram Values on Normal Dogs Six Months to One Year Old (Group II)

Animal Hospital Number	Percentage Protein Distribution per 100 ml. Plasma					Absolute Protein Distribution in gms. per 100 ml. Plasma					Total Plasma Protein in gms. per 100 ml. Plasma	
	Albumin	Alpha Glob.	Beta Glob.	Fibrinogen	Gamma Glob.	Albumin	Alpha Glob.	Beta Glob.	Fibrinogen	Gamma Glob.	Banco	Protometer
66- 8799	61	14	10	10	5	4.09	0.95	0.68	0.36	0.68	6.7	6.8
66- 8848	63	17	6	9	5	3.92	1.11	0.39	0.31	0.63	6.7	6.8
66-10844	54	15	13	13	5	3.09	0.84	0.77	0.28	0.77	5.8	5.7
66-11269	50	19	9	13	9	3.19	1.16	0.54	0.54	0.78	6.1	6.3
66-12207	52	16	11	12	9	3.37	1.02	0.67	0.57	0.73	6.1	6.6
66-12557	61	13	12	9	5	3.46	0.75	0.67	0.30	0.54	5.8	5.7
66-12780	60	15	9	10	6	4.02	1.01	0.60	0.40	0.67	6.7	6.7
66-13145	51	18	12	13	6	3.50	1.17	0.77	0.39	0.87	6.7	6.7
66-13410	49	16	12	19	4	3.26	1.02	0.75	0.27	1.20	6.7	6.3
66-13411	63	11	10	12	4	3.64	0.63	0.55	0.24	0.69	5.8	5.7
66-13418	57	15	6	13	9	3.80	0.99	0.42	0.56	0.83	6.7	6.5
66-14151	54	17	9	12	8	3.12	0.98	0.53	0.45	0.65	5.6	5.9
Mean	56.2	15.4	9.8	12.0	6.2							6.3[2]
S.D.[1]	4	2	2	3	2							

1. Standard deviation.

2. Mean of averaged total plasma proteins.

ESR and Plasma Cholesterol

The ESR determinations (Table 3) in this group of normal dogs were all within normal limits after one hour. The results obtained utilizing the Wintrobe tube and the Landau-Adams micro-sedimentation pipette were in close agreement.

The plasma total cholesterol values (Table 3) of this group were in the highest of the three normal dog groups. Four of the twelve dogs in this group were found to have plasma cholesterol values higher than normal (Schalm 1965). The ESR appeared to be unaffected by the increased cholesterol values in these animals.

Plasma Total Protein, Specific Gravity, Albumin-Globulin Ratios and Electrophoretic Analysis

The total plasma protein values (Table 4) determined by specific gravity and index of refraction agreed with those reported in the literature (Schalm 1965).

With the exception of one protein component the relative distribution (Table 4) was similar to those of the puppies in Group I (Table 2). A relative increase in albumin occurred at the expense of the alpha and beta globulins. A marked absolute increase was observed in albumin and gamma globulin.

In reviewing the relative protein distribution reported by other workers (Table 7), it was noted that disagreement existed concerning the alpha and beta globulins. Studies purportedly conducted on plasma failed to list fibrinogen values. It was felt that some reported variations existed due to differences in method of analysis; however, it was not likely

that complete reversal of alpha and beta globulin percentages took place as a result of technique alone. Discrepancies probably also occurred in interpretation of separations.

Some trends were indicated from the data (Table 4) obtained in this study of young normal dogs. The observed albumin values were higher slightly than those of the literature (Table 7); this was evidenced particularly on a relative basis, even though consistent throughout all groups of normal dogs. Absolute albumin values were in agreement with previous reports (Bloom 1960). The relative alpha globulins consistently were approximately 30% greater in value than beta globulins. Comparison with previously published investigations was difficult to assess. As previously mentioned, the literature reports indicated that the variation in relative amounts of alpha and beta globulins were somewhat dependent on the worker (Table 7). Fibrinogen values obtained in this study compared favorably with those reported by Bloom (1960). Gamma globulin values (Table 4) were found to be relatively low in comparison with other investigations (Table 7). It was impossible to determine the variation in exposure to antigenic stimuli in animals in this study and other investigations.

It was observed in this study that neither the alpha nor beta globulins were elevated in the four dogs with elevations in plasma cholesterol (Table 3).

Normal Dogs

Group III, Two - Six Years of Age

Routine Hematological Examination

Consistent agreement with values reported for mature dogs by Schalm (1965) was observed in the determination of hemoglobin concentration, packed cell volume, total red cell count and erythrocyte indices (Table 5). Similarly,

Table 5. Results of Erythrocyte Studies and Blood Chemistries on Normal Dogs Two to Six Years Old (Group III)

Animal Hospital Number	Hb[1]	PCV in %	Total RBC x 10^6	MCV[2]	MCH[3]	MCHC[4]	Uncorrected ESR in mm. Wintrobe 15	Wintrobe 30	Wintrobe 45	Wintrobe 60	Landsm-Adams Minutes 15	Landsm-Adams 30	Landsm-Adams 45	Landsm-Adams 60	A/G	Plasma Sp. Gr.	Plasma[5] Cholesterol
66-8628	15.7	45	5.550	71	25	34.9	0	0	0	0.5	0.2	0.2	0.5	0.5	0.97	1.0270	158
66-11265	15.9	48	5.210	82	27	33.1	0	0	0	0	0	0	0	0.5	1.85	1.0265	128
66-11266	15.2	45	5.140	78	26	33.8	0	0	0	3.	0	0	0	1.	1.61	1.0250	128
66-11267	13.6	42	4.740	80	26	32.4	0	0	2.	2.	0	0	0	1.	1.17	1.0240	111
66-11268	14.8	43	5.100	75	26	34.4	0	0	0	2.	0	1.	1.	2.	1.76	1.0265	211
66-12208	16.2	50	5.480	81	26	32.4	0	0	0	0	0	0	0	0	1.29	1.0280	122
66-12209	13.6	42	4.775	79	26	32.4	0	0	0	0	0	0	0	0	2.22	1.0235	279
66-12758	17.0	53	6.225	74	24	32.1	0	0	0	0	0	0	0	0	1.89	1.0265	148
66-13580	13.3	39	5.045	69	23	34.1	0	0	1.	2.	0	0	2.	4.	1.43	1.0255	122
66-13403	16.2	50	6.060	72	23	32.4	0	0	0	0	0	0	0	0	1.39	1.0267	255
66-14175	14.0	42	5.095	73	24	33.3	0	0	0	0	0	0	0	0	1.14	1.0267	466
Mean	15.0	45.4	5.311	75.8	25.1	33.2									1.49	1.0259	193.5

1. Hemoglobin concentration in gms. per 100 ml. blood.
2. Mean corpuscular volume in cubic microns.
3. Mean corpuscular hemoglobin in micromicrograms.
4. Mean corpuscular hemoglobin concentration in percent.
5. Total cholesterol in mg. per 100 ml. plasma.

Table 6. Total Plasma Protein and Electrophoretogram Values of Normal Dogs Two to Six Years Old (Group III)

Animal Hospital Number	Percentage Protein Distribution per 100 ml. Plasma					Absolute Protein Distribution in gms. per 100 ml. Plasma					Total Plasma Protein in gms. per 100 ml. Plasma	
	Albumin	Alpha Glob.	Beta Glob.	Fibrinogen	Gamma Glob.	Albumin	Alpha Glob.	Beta Glob.	Fibrinogen	Gamma Glob.	Benco	Protometer
66- 8628	42	15	20	16	7	2.72	0.99	1.32	1.08	0.49	6.8	6.4
66-11265	55	11	10	15	9	3.64	0.73	0.66	0.99	0.58	6.7	6.5
66-11266	58	11	12	9	10	3.56	0.68	0.74	0.55	0.62	6.1	6.2
66-11267	55	14	11	12	8	3.19	0.81	0.64	0.70	0.46	5.8	5.8
66-11268	56	15	11	12	6	3.78	1.03	0.70	0.77	0.42	6.7	6.7
66-12208	46	14	20	12	8	4.09	1.01	1.55	0.92	0.62	7.2	7.7
66-12209	62	14	11	11	2	3.54	0.83	0.62	0.62	0.14	5.6	5.9
66-12758	55	12	11	14	8	3.79	0.80	0.70	0.90	0.50	6.7	6.7
66-13580	50	12	12	16	10	3.02	0.75	0.75	1.00	0.61	6.3	5.8
66-13403	50	14	13	14	9	3.38	0.95	0.88	0.95	0.61	6.7	6.8
66-14175	45	18	9	14	14	3.11	1.19	0.64	0.91	0.91	6.7	6.8
Mean	53.2	13.7	12.8	13.1	8.3							6.5[2]
S.D.[1]	6	2	2	2	3							

1. Standard deviation.

2. Mean of averaged total plasma proteins.

Table 7. Relative Distribution of Normal Dog Plasma or Serum Proteins as Reported in the Literature.

Author	Literature Source	Plasma or Serum	Number of Dogs	Age	Relative Distribution* Albumin	Alpha	Beta	Fibrinogen	Gamma	Method of Separation
Ebel (1953)	Dimopoullos (1963)	Plasma	-	-	51.94	13.26	22.03	-	12.70	not available
Hahn (1956)	(same)	Plasma	7	Adult	45.9	24.53	23.7	-	4.8	moving boundary
Brueckner (1954)	Dimopoullos (1963)	Plasma	-	Adult	45.4	23.	12.1	14.4	5.1	moving boundary
Groulade and Groulade (1953)	Dimopoullos (1963)	Plasma	15	-	60.5	11.5	20.	-	7.6	moving boundary
deWael and Teunissen (1954)	Dimopoullos (1963)	Plasma	16	-	48.7	13.51	25.31	-	12.71	not available
Boguth (1953)	Dimopoullos (1963)	Plasma	20	-	53.5	13.8	20.4	-	12.3	not available
Engle (1961)	Putnam (1961)	Plasma	-	-	39.6	24.9	13.	13.3	9.3	moving boundary
Tomada (1963)	(same)	Serum	12	1 mo.	48.6	20.3	23.3	-	7.4	paper
Tomada (1963)	(same)	Serum	10	3 mo.	52.	17.5	22.9	-	7.6	paper
Tomada (1963)	(same)	Serum	5	6 mo.	51.1	16.2	22.7	-	7.5	paper
Tomada (1963)	(same)	Serum	12	1 yr.	48.4	15.8	22.4	-	13.4	paper
Tomada (1963)	(same)	Serum	7	5-9 yr.	44.8	15.2	23.8	-	16.2	paper
Tomada (1963)	(same)	Serum	4	10 yr.+	38.9	18.3	24.9	-	17.9	paper

*Relative distribution of proteins as reported by electrophoretic separation, Alpha, Beta and Gamma were reported globulin fractions.

agreement was found in total leukocyte count and relative and absolute distribution of leukocytes (Appendix Table 3) indicated by Schalm (1965).

ESR and Plasma Cholesterol

ESR values obtained from the blood of this group of normal dogs indicated that very little sedimentation occurred after one hour. In comparing the two methods of ESR determination, it was observed that agreement was fairly close until 2 mm. fall was observed in either the Wintrobe tube or Landau pipette. When sedimentation exceeded 2 mm. by one method or the other, discrepancies became apparent. A direct relationship between the two methods was not observed. Generally, faster sedimentation occurred in the Wintrobe tube than in the Landau pipette; however, this was not observed always.

Plasma cholesterol values (Table 5) for mature dogs averaged lower than the age group preceding it. The plasma cholesterol of one dog in this group was 466 mg./100 ml.; however, the sedimentation rate for this sample was not elevated. All other dogs of this group were considered to have plasma cholesterol values within the normal reported range (Bloom 1960).

Plasma Specific Gravity, Total Protein and Electrophoretic Analysis

The determinations of total protein (Table 6) by specific gravity and refractive index agreed with those reported in the literature Schalm (1965). They were similar to those obtained in the young adult dogs of Group II.

The electrophoretic separations (Table 6) were also similar to those of the previous group of dogs. The major change occurred in gamma globulin which increased both relatively and absolutely. This increase occurred primarily at the expense of albumin. The mean values of alpha globulin and beta globulin were similar in this group of dogs. This shift was due to an absolute decrease

of alpha globulin and a relative increase of beta globulin.

Relative albumin values obtained in this study were greater than those indicated in the literature (Table 7). This finding was not disturbing, as mean relative albumin values determined in all three groups of normal dogs were generally consistent while mean relative values for plasma albumin found in the literature ranged from 39.6% to 60.6% (Table 7). The reported determinations of alpha and beta globulins in the literature similarly have suggested variability (Table 7). In all groups of normal dogs relative alpha and beta globulins were noted to be more consistent in value than those reported in the literature.

Relative fibrinogen values compared favorably with those reported for normal dogs (Table 7). Absolute fibrinogen levels were within the normal range as proposed by Bloom (1960).

Relative gamma globulin values were low compared to the results indicated by most other investigators (Table 7). This observation was similarly observed in the preceding groups of dogs. The explanation for this result was difficult to determine. Although variable exposure levels to antigenic stimuli must be considered as a primary cause of this discrepancy, it was difficult to ascribe the entire pattern of inconsistency to this cause. The significance of other causes for these disagreements with previous reports was impossible to evaluate.

The plasma sample with the elevated cholesterol value had the highest absolute alpha globulin and one of the lowest beta globulin values of the group. This observation was not in agreement with a suggestion in the literature which associated increased cholesterol levels with increases in beta globulins (Wuhrman and Wunderly 1960).

Normal Cats

Group I, Two and Three Months of Age

The very small numbers in this group indicated the difficulty of obtaining an adequate amount of blood from a young kitten without exsanguinating it. The data were given this consideration, and that they might not be representative of this age group was conceded. Nevertheless, evaluations were attempted and general trends noted.

Routine Hematological Examination

Examination of the data (Table 8) obtained from the determinations of hemoglobin concentration, packed cell volume, total erythrocyte count, MCV, MCH, and MCHC indicated they were in agreement with those reported in the literature (Schalm 1965). Erythrocytes of kittens of this age have been reported by the same author to be slightly macrocytic and slightly hypochromic. This observation was confirmed in this study.

The examination of the total white cell counts (Appendix Table 4) and their relative and absolute distribution of leukocytes suggested that mild physiological leukocytosis influenced the results. This was considered to be the cause of the elevated total white blood cell count, since a relative and absolute increase in the number of neutrophils was observed. Schalm (1965) indicated that mild physiological leukocytosis in the cat was characterized by increased neutrophils.

Table 8. Results of Erythrocyte Studies and Blood Chemistries on Normal Cats Two and Three Months Old (Group I)

Animal Hospital Number	Hb[1]	PCV in %	Total RBC x 10^6	MCV[2]	MCH[3]	MCHC[4]	Uncorrected ESR in mm. Wintrobe Minutes 15	30	45	Landau-Adams Minutes 60	15	30	45	60	A/G	Plasma Sp. Gr.	Plasma[5] Cholesterol
Cat 3	9.2	29	5.100	51	16	31.7	4.	9.	16.	22.	0.	1.	5.	14.	0.74	1.0265	106
Cat 4	10.4	33	5.600	52	16	31.5	3.	6.	12.	16.	0.5	2.	7.	10.	0.63	1.0270	110
Cat 5	9.5	29	5.100	51	17	32.8	14.	30.	50.	62.	10.	27.	43.	50.	0.65	1.0265	76.5
Mean	9.7	30.3	5.260	51.3	16.3	32.0				33.				24.	0.69	1.0266	97.5

1. Hemoglobin concentration in gms. per 100 ml. blood.

2. Mean corpuscular volume in cubic microns.

3. Mean corpuscular hemoglobin in micromicrograms.

4. Mean corpuscular hemoglobin concentration in percent.

5. Total cholesterol in mg. per 100 ml. plasma.

Table 9. Total Plasma Protein and Electrophoretogram Values of Normal Cats Two and Three Months Old (Group I)

Animal Hospital Number	Percentage Protein Distribution per 100 ml. Plasma								Absolute Protein Distribution in gms. per 100 ml. Plasma							Total Plasma Protein in gms. per 100 ml. Plasma	
	Alb	Total Alpha Glob.	Alpha 1	Alpha 2	Alpha 3	Beta Glob.	Fibrinogen	Gamma Glob.	Alb	Alpha Glob.	Alpha 1	Alpha 2	Alpha 3	Beta Glob.	Fibrinogen	Gamma Glob.	Banco	Protometer
Cat 3	47	23	3	11	9	8	8	14	3.01	1.38	0.18	0.66	0.55	0.46	0.46	0.83	6.3	6.0
Cat 4	46	28	5	12	11	9	7	10	3.13	1.87	0.35	0.78	0.73	0.57	0.43	0.65	6.8	6.5
Cat 5	46	24	4	7	13	8	8	14	2.75	1.43	0.22	0.43	0.79	0.50	0.50	0.87	6.3	5.8
Mean	46.5	24.8	4.0	9.8	11.0	8.1	7.4	12.5										6.3[2]
S.D.	2	3	1	2	2	1	1	2										

1. Standard deviation.

2. Mean of averaged total plasma proteins.

ESR and Plasma Cholesterol

Flint et al. (1959) found that the blood from 21 normal cats had a mean uncorrected ESR value of 42.9 mm. in one hour. In her study the Wintrobe sedimentation tube was used. Mean hemoglobin concentration and mean packed cell volume values were reported to be within normal ranges. The sparsity of other available information on ESR in the cat was indicated by Schalm (1965): he stated, "No mention of the sedimentation test of cat's blood was found in the literature." He then suggested that the ESR values of canine blood be used as guidelines to determine the significance of ESR in the cat. Corrected ESR values greater than 10% of those for canine were suggested to be indicative of disease.

The ESR values obtained from determinations on the blood of the three kittens in Group I were erratic (Table 8 by both methods of determination. Values obtained by the Wintrobe tube ranged from 16 mm. to 62 mm. in one hour, while those of the Landau pipette ranged from 16 mm. to 50 mm. In comparing the results obtained by the two methods of ESR determination it was determined that values associated with the Landau microsedimentation pipette were approximately 30% less than those obtained utilizing the Wintrobe sedimentation tube.

Plasma cholesterol values (Table 8) were within the range of 75 to 150 mg./100 ml. serum reported to be normal for the cat (Bloom 1960).

Plasma Specific Gravity, Total Protein and Electrophoretic Separation

The total protein values (Table 9) obtained by specific gravity determination and refractive index were higher than reported in the literature for this age group. Groulade et al. (1965) determined the total serum protein

values in 15-45 day old cats to be 3.7 gm./100 ml. serum and in reviewing the results of other investigators reported that Otten (1954) found a mean total serum protein value in a one month old kitten to be 4.83 gm./100 ml. serum.

The method of electrophoretic separation utilized in this study permitted the separation of alpha globulins into three distinct fractions. This was expected since similar observations were indicated in the literature (Table 14). The relative distribution of proteins was in reasonably close agreement with those reported for this age of kitten (Table 14).

The major disagreement with other reported investigations was found in the interpretation of alpha and beta globulins.(Table 14) This finding has not discredited the previously reported data but calls attention to the liklihood of potential disagreement due to method of separation.

Normal Cats

Group II, Seven to Fifteen Months of Age

Data (Table 10) obtained from the determinations of hemoglobin concentration, packed cell volume, total erythrocyte count, MCV, MCH, and MCHC were consistent with those reported by Schalm (1965).

Examination of the data (Appendix Table 5) obtained from the determinations of total white cell count, relative and absolute distribution of leukocytes revealed that they were consistent with normal values as suggested by Schalm (1965). Physiological leukocytosis, although apparent in the laboratory examination of some cat blood, was not as readily obvious when the group values were examined.

Table 10. Results of Erythrocyte Studies and Blood Chemistries on Normal Cats Six months to Fifteen Months Old (Group II)

Animal Hospital Number	Hb[1]	PCV in %	Total RBC x 10^6	MCV[2]	MCH[3]	MCHC[4]	Uncorrected ESR in mm. Wintrobe Minutes 15	30	45	60	Landau-Adams Minutes 15	30	45	60	A/G	Plasma Sp. Gr.	Plasma Cholesterol
Sam	15.7	43	7.692	47	17	36.5	1.	5.	12.	20.	0	0	1.	2.	1.05	1.0295	160
Bacar	14.8	41	7.449	47	17	36.1	1.	5.	9.	10.	0	0	1.	2.	1.09	1.0295	149
Rapunsel	13.3	42	7.259	49	16	31.7	0	1.	3.	8.	0	0	0.5	2.	0.88	1.0295	121
Brand X	12.8	32	6.493	43	17	40.0	0	1.	2.	8.	0	0.5	0.5	1.	0.85	1.0280	121
Roscoe	14.0	39	7.835	42	15	35.9	0	0	1.	2.	0	0	0.5	2.	1.36	1.0280	121
Cat 6	12.6	40	6.810	50	16	31.5	2.	4.	7.	12.	0	0.5	0.5	1.	1.00	1.0270	40.4
Cat 7	12.2	36	6.430	48	16	33.9	0	2.	2.	4.	0	1.	1.	1.	1.50	1.0265	62.5
Cat 9	11.9	35	6.420	47	16	34.0	0	4.	6.	9.	0	2.	4.	10.	1.30	1.0270	67.
Cat 10	12.2	36	6.350	49	17	33.9	2.	6.	10.	16.	0	3.	5.	8.	0.90	1.0267	53.4
Cat 11	12.6	37	7.280	43	15	34.1	2.	4.	10.	18.	0	1.	1.	1.	0.87	1.0270	71.5
Cat 12	11.6	38	6.610	49	15	30.5	2.	5.	10.	16.	2.	3.	13.	15.	0.81	1.0270	49.
Cat 14	11.0	33	6.420	44	15	33.3	2.	3.	6.	10.	0	0	2.	4.	1.02	1.0255	92.

Table 10. (continued)

Animal Hospital Number	Hb[1]	PCV in %	Total RBC x 10^6	MCV[2]	MCH[3]	MCHC[4]	Uncorrected ESR in mm. Wintrobe Minutes 15	30	45	60	Landau-Adams Minutes 15	30	45	60	A/G	Plasma Sp. Gr.	Plasma[5] Cholesterol
Cat 15	11.9	37	6.750	47	15	32.2	4.	18.	28.	42.	0	1.	1.	4.	1.11	1.0270	86.
Cat 17	15.2	42	7.920	44	16	36.2	1.	4.	8.	12.	1.	2.	4.	5.	1.38	1.0295	95.
Mean	14.9	37.9	6.980	46.4	15.9	34.3				13.3				4.1	1.08	1.0277	92.1

1. Hemoglobin concentration in gms. per 100 ml. blood.

2. Mean corpuscular volume in cubic microns.

3. Mean corpuscular hemoglobin in micromicrograms.

4. Mean corpuscular hemoglobin concentration in percent.

5. Total cholesterol in mg. per 100 ml. plasma.

Table 11. Total Plasma Protein and Electrophoretogram Values of Normal Cats Six to Fifteen Months of Age (Group II)

Animal Hospital Number	Percentage Protein Distribution per 100 ml. Plasma								Absolute Protein Distribution in gms. per 100 ml. Plasma							Total Plasma Protein in gms. per 100 ml. Plasma	
	Alb	Total Alpha Glob.	Alpha 1	Alpha 2	Alpha 3	Beta Glob.	Fibrinogen	Gamma Glob.	Alb	Alpha Glob.	Alpha 1	Alpha 2	Alpha 3	Beta Glob.	Fibrinogen	Gamma Glob.	Banco	Protometer
Sam	56	17	1	6	10	8	6	13	4.37	1.29	0.08	0.44	0.77	0.62	0.46	0.96	7.7	7.7
Bacar	59	20	3	7	10	8	7	6	4.54	1.54	0.23	0.54	0.77	0.62	0.54	0.46	7.7	7.7
Rapunsel	52	19	3	8	8	9	8	12	4.03	1.47	0.23	0.62	0.62	0.70	0.62	0.93	7.7	7.8
Brand X	57	18	1	8	9	9	6	10	3.34	1.29	0.07	0.57	0.64	0.64	0.43	0.71	7.2	7.0
Roscoe	64	16	2	8	6	7	5	8	4.51	1.13	0.14	0.56	0.42	0.44	0.35	0.56	7.2	6.9
Cat 6	55	17	1	6	10	9	7	12	3.69	1.14	0.07	0.40	0.67	0.60	0.47	0.80	6.8	6.6
Cat 7	66	15	2	6	7	9	5	5	4.36	0.99	0.13	0.40	0.46	0.59	0.33	0.33	6.7	6.5
Cat 9	61	14	2	5	7	9	6	10	4.09	0.94	0.13	0.34	0.47	0.60	0.40	0.67	6.8	6.6
Cat 10	54	20	2	8	10	10	6	10	3.56	1.32	0.13	0.53	0.66	0.66	0.40	0.66	6.7	6.5
Cat 11	53	21	1	9	11	11	7	8	3.52	1.40	0.07	0.60	0.73	0.73	0.47	0.53	6.8	6.5
Cat 12	59	17	2	7	8	10	5	9	3.98	1.15	0.14	0.47	0.54	0.68	0.34	0.61	6.8	6.7
Cat 14	51	20	2	9	9	11	6	12	3.19	1.25	0.13	0.56	0.56	0.69	0.38	0.75	6.3	6.2

Table 11. (continued)

Animal Hospital Number	Percentage Protein Distribution per 100 ml. Plasma								Absolute Protein Distribution in gms. per 100 ml. Plasma								Total Plasma Protein in gms. per 100 ml. Plasma	
	Alb.	Total Alpha Glob.	Alpha 1	Alpha 2	Alpha 3	Beta Glob.	Fibrinogen	Gamma Glob.	Alb.	Alpha Glob.	Alpha 1	Alpha 2	Alpha 3	Beta Glob.	Fibrinogen	Gamma Glob.	Banco	Protemeter
Cat 15	57	19	2	6	11	10	6	8	3.82	1.27	0.13	0.40	0.74	0.67	0.40	0.54	6.8	6.2
Cat 17	62	14	1	6	7	12	7	5	4.71	1.06	0.08	0.46	0.53	0.91	0.53	0.61	7.7	7.5
Mean	56.9	17.6	1.8	7.1	8.8	9.4	6.2	9.1										6.5[2]
S.D.	5	2	7	1	2	1	1	2										

1. Standard deviation.

2. Mean of averaged total protein values.

ESR and Plasma Cholesterol

The results (Table 10) obtained by the two methods of ESR determination were erratic. The range of mm. fall per hour as determined utilising the Wintrobe tube ranged from 2 mm. to 42 mm. while a range of 1 mm. to 15 mm. fall was observed in employing the Landau-Adams microsedimentation pipette. The Wintrobe tube resulted in the more rapid sedimentation rate in the majority of this group. No consistent pattern of relationship was found, as in four of the fourteen samples examined, sedimentation in the Wintrobe tube was 10 times that observed in the Landau pipette. In one sample the Landau pipette sedimentation exceeded that found in the Wintrobe tube. Mean one hour values obtained utilising the Landau-Adams pipette were 25% of the mean values determined using the Wintrobe tube. Plasma total cholesterol values (Table 10) were determined to be normal according to those reported by Blood (1960).

Specific Gravity, Total Plasma Protein and Electrophoretic Separations

The total plasma protein values (Table 11) obtained by the measurement of specific gravity and refractive index were similar to those reported by Bloom (1960) and Groulade et al. (1965). The absolute increase in total plasma protein over the kittens in Group I was not remarkable. Several changes (Table 11) in the relative distribution of proteins were apparent when the results obtained from analysis of Group I and Group II were compared (Table 9). The plasma of young cats of Group II contained more albumin on both a relative and absolute basis. Similarly, beta globulins were increased in the plasma of young cats. On the other hand gamma globulin decreased slightly on both a relative and absolute basis. Fibrinogen levels decreased on a relative basis, but essentailly remained unchanged in absolute value.

The differences found among the alpha globulin fractions between the two groups also merited attention. The relative distribution of the alpha 1 fraction of young cats was only half that of the kittens. Alpha 2 and alpha 3 globulins also decreased; however, these differences were not pronounced in either relative or absolute comparison.

Relative albumin values obtained in this study were higher than those reported by Groulade et al. (1965) in similarly age cats (Table 14). Beta globulin values obtained in this study were slightly lower than those reported by Groulade et al. (1965). The decrease of alpha 1 globulins with age was suggested from the data of Groulade et al. (1965), although the trend he observed was not as pronounced as data from this study indicated. Groulade et al. (1965) also reported slight decreases of alpha 2 and alpha 3 globulins with increasing age.

Normal Cats

Group III, Two - Six Years of Age

Routine Hematological Examination

The hematologic values (Table 12) of hemoglobin concentration, packed cell volume, total erythrocyte count, MCV, MCH, MCHC were in agreement with those reported by Schalm (1965).

Similarly total white cell counts and relative and absolute distributions (Appendix Table 6) were in agreement with those reported by Schalm (1965). The blood of one cat in this group contained excessive eosinophils both on a relative and absolute basis; as this cat was clinically normal and other hematological findings did not suggest disease, this was regarded as an incidental finding.

Table 12. Results of Erythrocyte Studies and Blood Chemistries on Normal Cats Two to Six Years Old (Group III)

Animal Hospital Number	Hb[1]	PCV in %	Total RBC x 10^6	MCV[2]	MCH[3]	MCHC[4]	Uncorrected ESR in mm. Wintrobe Minutes 15	30	45	60	Landau-Adams Minutes 15	30	45	60	A/G	Plasma Sp. Gr.	Plasma[5] Cholesterol
66- 8072	13.6	43	7.280	50	16	31.6	1	2	8	13	0.2	0.5	0.5	1	1.06	1.0300	95
66-10637	11.2	35	6.015	51	16	32.0	1	5	9	12	0.5	2	4	10	0.98	1.0280	95
Mary Ann	14.0	39	6.278	54	19	35.9	0	1	3	8	0	0.5	1	2	1.02	1.0289	81
Peggy	13.3	39	6.835	49	17	34.1	1	4	9	14	0	0.5	1.5	6	0.98	1.0260	67
Spook	12.8	37	5.428	60	21	34.6	2	9	30	46	0	0.5	5	7	0.84	1.0280	110
Cat 1	11.2	35	5.965	51	16	32.0	9	22	36	50	0	2	7	23	0.82	1.0272	53.4
Cat 2	10.8	33	6.200	46	15	32.7	2	4	10	14	0	0	4	7	1.00	1.0270	95
Cat 8	13.0	41	7.320	47	15	31.7	0	0	0	0	0	0	2	4	1.04	1.0272	67
Cat 13	11.0	33	6.700	42	14	33.3	2	4	8	14	0	2	6	8	1.00	1.0270	85.5
Cat 16	13.3	33	7.210	39	16	40.3	2	32	53	56	0	2	8	23	0.76	1.0290	44.6
Mean	12.4	36.8	6.523	48.9	16.5	33.8				22.7				9.1	0.95	1.0280	79.4

1. Hemoglobin concentration in gms. per 100 ml. blood.
2. Mean corpuscular volume in cubic microns.
3. Mean corpuscular hemoglobin in micromicrograms.
4. Mean corpuscular hemoglobin concentration in percent.
5. Total cholesterol in mg. per 100 ml. plasma.

Table 13. Total Plasma Protein and Electrophoretogram Values of Normal Cats Two to Six Years of Age (Group III)

Animal Hospital Number	Percentage Protein Distribution per 100 ml. Plasma								Absolute Protein Distribution in gms. per 100 ml. Plasma								Total Plasma Protein in gms. per 100 ml. Plasma	
	Alb.	Total Alpha Glob.	Alpha 1	Alpha 2	Alpha 3	Beta Glob.	Fibrinogen	Gamma Glob.	Alb.	Alpha Glob.	Alpha 1	Alpha 2	Alpha 3	Beta Glob.	Fibrinogen	Gamma Glob.	Benco	Protometer
66- 8072	55	16	1	4	11	9	9	11	4.37	1.27	0.08	0.32	0.87	0.72	0.72	0.87	7.9	8.0
66-10637	56	20	3	6	11	9	7	8	3.95	1.41	0.21	0.42	0.78	0.63	0.49	0.56	7.2	6.9
Mary Ann	54	15	1	7	7	10	8	13	4.00	1.11	0.07	0.52	0.52	0.74	0.59	0.96	7.5	7.3
Peggy	55	17	2	5	10	9	6	13	3.49	1.08	0.13	0.32	0.64	0.57	0.38	0.83	6.5	6.2
Spook	49	17	2	5	10	9	10	15	3.55	1.23	0.15	0.36	0.73	0.65	0.73	1.09	7.2	7.3
Cat 1	47	16	1	5	10	9	12	16	3.13	1.06	0.07	0.33	0.67	0.60	0.80	1.06	6.8	6.5
Cat 2	55	14	2	7	5	4	4	23	3.69	0.94	0.13	0.47	0.34	0.27	0.27	1.54	6.8	6.6
Cat 8	57	17	3	7	7	10	5	11	3.82	1.14	0.20	0.47	0.47	0.67	0.34	0.74	6.8	6.6
Cat 13	57	19	3	8	8	11	5	8	3.85	1.28	0.20	0.54	0.54	0.74	0.34	0.54	6.8	6.7
Cat 16	45	13	2	5	6	10	9	23	3.29	0.95	0.15	0.37	0.44	0.73	0.66	1.68	7.4	7.2
Mean	53	16.4	1.9	5.9	8.5	9.0	7.5	14.1										7.0[2]
S.D.[1]	4	2	1	1	2	2	2	5										

1. Standard deviation.

2. Mean of averaged total plasma proteins.

Table 14. Relative Distribution of Normal Cat Plasma or Serum Proteins as Reported in the Literature.

Author	Literature Source	Plasma or Serum	Number of Cats	Age	Sex	Percentage Distribution* Alb.	Total Alpha	Alpha 1	Alpha 2	Alpha 3	Beta Glob.	Fibrinogen	Gamma Glob.	Method of Separation
Moore (1945)	(same)	Serum	1	4 day	-	46	37	12	14	14	12	-	5.	moving boundary
Moore (1945)	(same)	Serum	2	Adult	-	35.5	22.5	NS[1]	22.5	NS[1]	12.5	-	29.5	moving boundary
Deutsch (1945)	(same)	Plasma	4	Adult	-	41.1	33.	8.1	20.2	4.7	8.7	5.2	12.5	moving boundary
Causse-Vails (1961)	Groulade et al.(1965)	Serum	6	1 mo.	-	38.6	21.6	4.5	10.0	7.1	29.8	-	10.	paper
Causse-Vails (1961)	Groulade et al.(1965)	Serum	8	Adult	-	38.	25.6	1.3	7.7	16.6	16.8	-	20.5	paper
Antonini (1952)	Groulade et al.(1965)	Serum	63	Adult	-	40.	23.4	2.6	8.4	12.4	12.4	-	24.2	paper
Wilde (1957)	Groulade et al.(1965)	Serum	55	various	-	50.6	24.1	6.	3.7	14.4	12.8	-	16.8	paper
Groulade et al. (1965)	(same)	Serum	11	1 day	both	42.7	22.8	4.9	17.9	NS[2]	13.2	-	21.3	paper
Groulade et al. (1965)	(same)	Serum	10	15-45 days	both	46.1	30.	7.4	22.6	NS[2]	12.9	-	11.0	paper

Table 14. (continued)

Author	Literature Source	Plasma or Serum	Number of Cats	Age	Sex	Percentage Distribution* Alb.	Total Alpha	Alpha 1	Alpha 2	Alpha 3	Beta Glob.	Fibrinogen	Gamma Glob.	Method of Separation
Groulade et al. (1965)	(same)	Serum	11	6-9 mo.	both	41.	27.1	5.2	21.9	NS[2]	10.7	-	21.1	paper
Groulade et al. (1965)	(same)	Serum	12	2-3 yr.	both	35.7	24.8	5.6	19.2	NS[2]	14.0	-	25.6	paper

*Relative distribution of protein fractions as reported in per cent Alpha, Beta and Gamma globulin.

[1]NS indicates that all values were reported as Alpha 2 globulin.

[2]NS indicates that Alpha 2 and Alpha 3 globulins were not differentiated.

ESR and Total Plasma Cholesterol

The ESR values again indicated that this test is erratic in normal cat blood. The range of settling which occurred in one hour as determined by the Wintrobe sedimentation tube was observed to be 0 mm. to 50 mm.; the range for the Landau pipette was similarly variable, 1 mm. to 23 mm. In only two of ten samples in this group were the ESR results obtained from the Wintrobe tube and Landau pipette similar. The results obtained with the Wintrobe tube generally exceeded those obtained with the microsedimentation pipette. In one sample the Landau pipette value exceeded that found using the Wintrobe tube. The mean values obtained with the Landau pipette were less than 50% of the mean value obtained using the Wintrobe tube. Mean values of this age group of cats were higher than those of the immature cats but less than those of the three kittens in Group I.

The total plasma cholesterol values (Table 12) were the lowest among the three groups of normal cats and were within the normal distribution range reported by Bloom (1960).

Specific Gravity, Total Protein and Electrophoretic Separations

The total protein values (Table 13) based on differences in specific gravity and refractive index were consistent with those reported by Bloom (1960). They were not appreciably different from total protein values of the immature cats of Group II. Total protein values for this age group agreed with those indicated by Groulade et al. (1965).

The relative albumin protein component (Table 13) was slightly decreased in this older group of cats. In absolute distribution albumin appeared to have changed little. With the exception of gamma globulin, slight changes from the relative and absolute distribution values of Group II were found

when remaining protein fractions were compared. Gamma globulin increased with age both in relative and absolute distribution. When comparing the results of other investigators (Table 14) with data obtained in this study, discrepancies similar to those described as occurring in the preceding group were observed. Albumin levels determined in this study were higher than those described by other investigators and gamma globulins were markedly lower than those reported by Groulade et al. (1965).

Diseased Dogs

Group I, Four Months - One Year of Age

Relationship of Routine Hematological Examination, Sedimentation Rates and Plasma Cholesterol Values to Normal Dogs of Comparable Age

In addition to signs of clinical disease, all dogs in this class reflected presence of illness in hematological examinations and ESR values after correction for anemia. ESR values determined by the Wintrobe tube which exceeded those reported for corresponding packed cell volume by Schalm (1965) were considered. Selecting those dogs in which anemia was determined to be of little clinical significance reduced the influence of anemia in ESR determinations.

All dogs in Group I reflected disease in the relative and absolute distribution of leukocytes even though total white cell counts were within normal limits (Appendix Table 7). The leukocytic response to illness suggested subacute or chronic duration rather than acute response in most instances. A severe relative and absolute lymphopenia was observed in blood samples from two animals.

Erythrocyte sedimentation rates (Table 15) were elevated if corrected for anemia (Schalm 1965). The results obtained from utilization of the

Table 15. Results of Erythrocyte Studies and Blood Chemistries on Diseased Dogs Four Months to One Year of Age (Group I)

Animal Hospital Number	Hb[1]	PCV in %	Total RBC x 10^6	MCV[2]	MCH[3]	MCHC[4]	Uncorrected ESR in mm. Wintrobe Minutes 15	30	45	60	Landau-Adams Minutes 15	30	45	60	A/G	Plasma Sp. Gr.	Plasma[5] Cholesterol
66-13582	15.2	48	6.655	62	20	31.7	0	0.5	6.	22.	0.5	1.	1.5	1.5	0.74	1.0289	203
66-13904	14.0	44	4.952	79	25	31.8	0.5	3.	18.	31.	1.5	3.	5.	7.	1.16	1.0270	148
66-13907	15.9	50	5.380	82	26	31.8	0	0.5	2.	2.	0.5	0.5	0.5	1.	0.72	1.0260	251
66-13911	18.0	50	6.230	70	25	36	0	0	1.	2.	0	0	0	0.5	0.70	1.0235	251

1. Hemoglobin concentration in gms. per 100 ml. blood.

2. Mean corpuscular volume in cubic microns.

3. Mean corpuscular hemoglobin in micromicrograms.

4. Mean corpuscular hemoglobin concentration in percent.

5. Total cholesterol in mg. per 100 ml. plasma.

Wintrobe tube generally exceeded those found in the Landau pipette; however, no consistent proportional discrepancy was observed. Disagreement was pronounced as increased settling was found in the Wintrobe tube. In one of the four samples the dissimilarity was pronounced as the Wintrobe tube ESR result was 22 mm. and the Landau pipette result was 1.5 mm.

Plasma cholesterol values (Table 15) were not in excess of normal and were not considered as likely causes of the increased ESR values found in this group.

Total Proteins and Electrophoretic Separations

Values obtained by the two methods of total protein analysis were similar to values obtained from normal dogs of comparable age (Table 4). Attempts to correlate increased ESR with shifts in protein distributions were moderately successful. Although albumin values were consistently decreased in both relative and absolute distribution, a proportional relationship between these changes and increased ESR was not found. Elevations in fibrinogen and alpha globulin values were consistently noted when ESR was increased. Beta globulins were relatively increased in two of the four dogs of this group. There was no apparent relationship between beta globulin and increased ESR.

When comparing the electrophoretic separations of this diseased dog group (Table 16) with those of normal dogs of comparable age, Group II (Table 4) differences were observed. The obvious difference was found to have occurred in the relative and absolute value of albumin. A very marked decrease in relative and absolute albumin values was observed in most of the plasma samples of diseased dogs in Group I. This observation was confirmed in the reduction of the ratio of albumin to globulin (A/G). Plasma levels of alpha globulins were elevated in the diseased dogs. Beta globulins were not con-

Table 16. Total Plasma Protein and Electrophoretogram Values of Diseased Dogs Four Months to One Year of Age (Group I)

Animal Hospital Number	Percentage Protein Distribution per 100 ml. Plasma					Absolute Protein Distribution in gms. per 100 ml. Plasma					Total Plasma Protein in gms. per 100 ml. Plasma	
	Albumin	Alpha Glob.	Beta Glob.	Fibrinogen	Gamma Glob.	Albumin	Alpha Glob.	Beta Glob.	Fibrinogen	Gamma Glob.	Banco	Protometer
66-13582	35	20	12	18	15	2.68	1.53	0.92	1.38	1.15	7.5	7.8
66-13904	44	21	9	18	8	3.04	1.45	0.62	1.24	0.55	6.8	7.0
66-13907	34	31	9	19	7	2.04	1.86	0.54	1.14	0.42	6.0	6.0
66-13911	32	24	17	12	5	1.78	1.33	0.94	0.67	0.28	5.6	5.5
Mean	35	24	12	17	8	2.39	1.54	0.76	1.11	0.60		

sistently elevated from normal values. Gamma globulin was elevated both on relative and absolute comparison, a finding that suggested chronicity of the disease. The amount of variation was inconsistent and was not observed to occur with the constant magnitude associated with alpha globulin fraction increases. The relative fibrinogen level was moderately increased in level while absolute values determined for fibrinogen appeared to be almost double those determined for normal dogs of comparable age. The range of total protein values in this group extended over a wider span than those of comparably aged normal dogs (Group II, Normal Dogs, Table 4).

Diseased Dogs

Group II, Two - Six Years of Age

Relationship of Routine Hematological Examination Sedimentation Rates and Plasma Total Cholesterol Values to Normal Dogs of Comparable Age

The absolute leukocyte distribution suggested disease in nine of the ten dogs in this group (Appendix Table 8). The leukocytic response was more characteristic of subacute or chronic illness in all but one animal.

As was described for the first group of diseased dogs, ESR values show an inconsistency between methods of determination (Table 17). The result obtained in one sample utilizing the Wintrobe tube was 32 mm.; the microsedimentation pipette on the same sample indicated a fall of only 2 mm. In eight of the ten samples the ESR was greater when observed in the Wintrobe tube. In two samples, sedimentation occurred at a faster rate in the Landau pipette. The explanation for this deviation was not readily apparent. The possibility existed that the microsedimentation pipette was not truly vertical, even though this was carefully checked. In only one dog in this group was total cholesterol value elevated (Table 17).

Table 17. Results of Erythrocyte Studies and Blood Chemistries on Two to Seven Year Old Diseased Dogs (Group II)

Animal Hospital Number	Hb[1]	PCV in %	Total RBC x 10^6	MCV[2]	MCH[3]	MCHC[4]	Uncorrected ESR in mm. Wintrobe Minutes 15	30	45	60	Landau-Adams Minutes 15	30	45	60	A/G	Plasma Sp. Gr.	Plasma[5] Cholesterol
Blaugh D	14.4	41	6.135	67	23	35.1	2	4	8	10	0.5	3	4	5	0.73	1.0240	154
66-13723	12.6	37	5.924	67	21	33.7	4	20	34	46	0.5	2	4	10	0.73	1.0270	209
66-13821	15.2	44	4.750	83	29	34.5	2	12	30	38	0.5	2	2	3	0.75	1.0250	154
66-13906	13.6	44	5.480	80	25	30.9	1	9	20	28	2	10	21	35	0.76	1.0290	126
66-14314	15.7	48	5.830	72	24	32.7	2	10	23	32	0	0.5	1.0	2	0.88	1.0310	334
66-14321	18.3	52	5.550	83	29	35.2	0	0	8	12	0	1	2	4	1.03	1.029	184
66-14328	16.2	47	5.037	83	29	34.5	0	2	4	8	0	1	2	2	1.33	1.0250	160
66-14342	16.2	50	5.650	78	25	32.4	0	0.5	2	8	0	9	12	14	0.95	1.0290	160
66-14414	17.6	54	6.120	77	25	32.6	0	0	2	4	0	0	1	2	0.97	1.0289	44.6
66-14836	12.2	35	4.610	68	24	34.9	2	10	19	26	1	3	9	18	1.13	1.0270	110

1. Hemoglobin concentration in gms. per 100 ml. blood.

2. Mean corpuscular volume in cubic microns.

3. Mean corpuscular hemoglobin in micromicrograms.

4. Mean corpuscular hemoglobin concentration in percent.

5. Total cholesterol in mg. per 100 ml. plasma.

Relationship of Total Plasma Protein, Electrophoretic Analysis Between Diseased Dogs and Normal Dogs of Comparable Age Group

Total proteins (Table 18) obtained by the two methods of determination were in close agreement with normal values (Table 6). Variations were found in the total protein values among diseased dogs.

The albumin-globulin ratio and electrophoretic separation (Table 18) revealed relative and absolute decreases in plasma albumin in diseased dogs. These findings are associated with chronic disease. Fibrinogen levels were increased when compared with the relative and absolute mean values of normal mature dogs (Group III, Table 6). In one dog an absolute fibrinogen increase was not observed; in this animal the alpha globulins were double the mean alpha percentage found in the normal dogs of comparable age. Mean relative and absolute gamma globulin values were increased slightly over those determined for normal dogs of this age.

Intriguing differences occurred in the alpha and beta globulins, particularly in the relationship between these two globulin fractions. In five of the ten plasma samples of this group, relative alpha globulins were elevated from the mean alpha globulin values of normal dogs while the beta globulin results resembled normal beta values. The plasma sample which contained an elevated cholesterol value was found in this group. One plasma sample had increased proportionally in both the alpha and beta globulins. In three plasma samples beta globulin levels were greater than alpha globulin values, and in two of the three samples relative alpha globulins were below the relative mean alpha of normal dogs of comparable age. These variations made it difficult to associate alpha-beta globulin relationship with ESR elevation.

Relative and absolute decreases in albumin were found when increased

Table 18. Total Plasma Protein and Electrophoretogram Values of Diseased Dogs Two to Six Years of Age (Group II)

Animal Hospital Number	Percentage Protein Distribution per 100 ml. Plasma					Absolute Protein Distribution in gms. per 100 ml. Plasma					Total Plasma Protein in gms. per 100 ml. Plasma	
	Albumin	Alpha Glob.	Beta Glob.	Fibrinogen	Gamma Glob.	Albumin	Alpha Glob.	Beta Glob.	Fibrinogen	Gamma Glob.	Biuret	Protometer
Blaugh D	32	23	12	13	9	1.89	1.36	0.71	0.77	0.53	5.8	6.0
66-13723	30	21	13	20	7	2.04	1.03	0.88	1.36	0.48	6.8	6.8
66-13821	33	16	18	23	10	2.34	1.14	1.28	1.63	0.71	7.1	7.1
66-13906	35	10	29	19	7	2.61	0.75	2.16	1.42	0.52	7.4	7.5
66-14314	36	23	12	22	6	2.99	1.91	1.00	1.83	0.50	8.2	8.4
66-14321	41	19	13	19	8	3.03	1.41	0.96	1.41	0.59	7.4	7.4
66-14328	48	8	19	16	9	3.38	0.56	1.34	1.13	0.63	7.1	7.0
66-14342	39	18	16	20	7	2.89	1.33	1.18	1.48	0.52	7.4	7.4
66-14414	37	13	11	25	14	2.85	1.00	0.85	1.93	1.08	7.7	7.7
66-14836	43	19	12	19	7	2.86	1.26	0.80	1.26	0.47	6.8	6.5
Mean	37.4	17	15.5	19.6	8.4							

ESR was observed; however, no relationship was indicated. Elevations in alpha globulin levels were associated with increased ESR values but a distinct relationship was not suggested. Increased fibrinogen levels, particularly on an absolute basis seemed to be crudely, related with increased ESR; only one exception to this finding was observed. There was no relationship associated with increased values of beta and gamma globulin and increased sedimentation of erythrocytes.

Diseased Dogs

Group III, Eight - Eleven Years of Age

Relationship of Hematological Determinations, ESR and Total Plasma Cholesterol to Normal Dogs.

Unfortunately, blood was not available from normal dogs in this age group during the period of study. Results obtained from the study of older diseased dogs was compared with mature young dogs as reasonable flexibility was assumed in the interpretation.

The determination of total leukocyte count (Appendix Table 9) and the relative and absolute distribution of white cells suggested that the duration of illnesses was chronic. A slight absolute neutrophilia was present in all samples. The methods employed to determine ESR values continued to disagree (Table 19). Millimeter fall obtained in the Wintrobe tube consistently exceeded that observed in the Landau microsedimentation pipette. The disagreement was not always proportional.

One of the plasma samples in this group contained elevated levels of total plasma cholesterol(Table 19).

Table 19. Results of Erythrocyte Studies and Blood Chemistries on Diseased Dogs Eight to Eleven Years Old (Group III)

Animal Hospital Number	Hb[1]	PCV in %	Total RBC x 10^6	MCV[2]	MCH[3]	MCHC[4]	Uncorrected ESR in mm. Wintrobe Minutes 15	30	45	60	Landau-Adams Minutes 15	30	45	60	A/G	Plasma Sp. Gr.	Plasma[5] Cholesterol
66-10454	15.7	45	5.155	78	27	34.8	1	3	12	22	0.5	0.5	0.5	1.0	0.72	1.0370	126
66-13910	13.6	43	4.850	79	25	31.6	0.5	2	5	8	0.5	0.5	1.5	2.	0.44	1.0315	372
66-14514	17.0	51	6.350	69	23	33.3	0	4	8	16	2	3	4	4	0.66	1.0310	116

1. Hemoglobin concentration in gms. per 100 ml. blood.

2. Mean corpuscular volume in cubic microns.

3. Mean corpuscular hemoglobin in micromicrograms.

4. Mean corpuscular hemoglobin concentration in percent.

5. Total cholesterol in mg. per 100 ml. plasma.

Total Proteins, Alpha Globulin Ratio, and Electrophoretic Separation

Tomada (1963) indicated that several changes in relative distribution of serum protein components occurred in older normal dogs (Table 7). Moderate decrease in relative albumin values was seen in older dogs. He reported slight increases in relative values of alpha, beta and gamma globulins as age progressed (Table 20).

Fibrinogen values (Table 20) obtained in this study were consistently elevated both in relative and absolute distribution. Albumin levels were decreased on a relative comparison and slightly decreased in absolute value. This finding was further evidenced by the marked shift in A/G. Levels of alpha and beta globulins were increased in this group. The gamma globulin values were increased slightly. The gamma globulin values of this group did not reflect the chronicity indicated by other hematologic examinations, namely the leukocytic response.

Increased rates of erythrocyte sedimentation (Table 19) appeared to be related to increased fibrinogen levels (Table 20). The decrease in albumin was inconsistent with accelerated ESR. No relationship was observed between elevations in beta or gamma globulins and increased ESR. Alpha globulins played an insignificant role in the increased sedimentation of erythrocytes of this group as these values did not differ appreciably from those of Group II normal dogs of comparable age (Table 5).

Upon electrophoretic analysis the sample which contained excessive quantities of plasma cholesterol contained increased amounts of beta globulin. It was felt that increased ESR in this sample was not due to the elevated cholesterol as fibrinogen values were elevated also.

Table 20. Total Plasma Proteins and Electrophoretogram Values for Diseased Dogs Eight to Eleven Years Old (Group III)

Animal Hospital Number	Percentage Protein Distribution per 100 ml. Plasma					Absolute Protein Distribution in gms. per 100 ml. Plasma					Total Plasma Protein in gms. per 100 ml. Plasma	
	Albumin	Alpha Glob.	Beta Glob.	Fibrinogen	Gamma Glob.	Albumin	Alpha Glob.	Beta Glob.	Fibrinogen	Gamma Glob.	Banco	Protometer
66-10454	33	14	16	21	16	2.74	1.16	1.33	1.74	1.34	8.2	8.4
66-13910	24	23	19	21	13	2.05	1.97	1.62	1.80	1.11	8.4	8.7
66-14514	31	11	15	22	21	2.57	0.91	1.25	1.83	1.74	8.4	8.2
Mean	29	16	16	21.5	16							

CONCLUSIONS

Normal Dogs

Several general conclusions were indicated from the results obtained in this study. Constituents of normal dog blood undergo significant changes as the animal grows older. These changes included decreased erythrocyte size, increased erythrocyte numbers with a resultant increase in packed cell volume. Lymphocytes decreased in total numbers until adult-hood values were attained.

The ESR data suggested that little sedimentation occurred in normal puppy blood. Studies in older dogs indicated that this persisted throughout the life of the animal. The ESR obtained by the Wintrobe tube and Landau microsedimentation pipette compared favorably on normal dogs. Plasma cholesterol values obtained were increased in the six month to one year old dogs, then decreased slightly in older dogs. These studies indicated that total plasma proteins were increased in dogs up to six years of age. The results obtained by determination of total protein using the Banco Density Gradient and Serum Protometer compared favorably in all normal dogs. Marked absolute increases of albumin occurred in mature dogs when compared with puppies. Gamma globulins continued to increase with progressing age. Only slight increases were observed in alpha and beta globulins with increasing age. Beta globulins increased with age at a slightly faster rate than alpha globulin until, in older dogs, the distribution of the alpha and beta fractions was similar. Fibrinogen levels were found to be constant in value throughout all age groups of normal dogs.

Normal Cats

The MCV of the red blood cells of kittens decreased when compared with older cats. Erythrocytes increased in numbers with age; this change was related to increased hemoglobin concentration and packed cell volume. Total white cell counts and distributions reflected the influence of physiological leukocytosis. This phenomenon, even though predominantly observed in young kittens, suggested that multiple determinations were indicated in all groups of cats.

Sedimentation rate determinations in all groups of cats were erratic, indicating that this test should not be used as a diagnostic aid to clinical feline medicine until additional normal values have been reported and evaluated. ESR values utilizing the Wintrobe tube and Landau microsedimentation pipette were in poor agreement when examining cat blood. The small internal diameter of the Landau microsedimentation pipette probably influenced the irregular results. Mean plasma cholesterol values decreased with age in cats.

Total plasma protein increased with age in normal cats. This increase was primarily due to increase in albumin and gamma globulin fractions. Total plasma protein values determined by specific gravity or refractive index compared favorably. All alpha globulin values examined in this study decreased until maturity. Alpha 2 globulin values continued to decrease with advancing age, while alpha 1 and alpha 3 globulins became stabilized. Beta globulins increased slightly as kittens grew older. Fibrinogen levels were consistent throughout all ages of cats in this study.

Diseased Dogs

In all three groups of diseased dogs, single leukocyte examinations were not felt to reflect adequately the status of the animal. The leukocyte response associated with chronic illness failed to mirror the changes found in electrophoretic separation of plasma.

The use of the Landau microsedimentation pipette was found not to be justified as it often failed to correlate with increases found using the Wintrobe tube. The suggestion that sedimentation tubes of bore diameter smaller than 2.5 mm. produce decreased and irregular ESR results was confirmed in this study.

Plasma total cholesterol values were not related to accelerated ESR.

Total protein values were more variable in diseased dogs than normal dogs. This finding was confirmed in all three groups of diseased dogs. Although no correlation between elevated ESR and albumin globulin ratios was indicated, the A/G consistently reflected illness. There was good evidence that the determination of A/G ratio indicated the chronicity of the disease when the leukocyte count and ESR did not adequately emphasize the duration of the problem. The conclusion from this observation was that the A/G ratio might be employed more often as a significant diagnostic aid, particularly when electrophoretic analysis was not possible.

Electrophoretic separations indicated that some fractions of plasma protein more strongly influenced ESR than others. Fibrinogen levels were increased in essentially all cases where ESR was increased. There was less relationship between increased ESR results and alpha globulins. Decreased albumin values and increased ESR apparently were not related. The observed variations in beta globulin values did not influence ESR. Similarly, gamma

globulin was assigned an insignificant role in affecting the settling of erythrocytes.

In comparing the results from normal and diseased dogs it was observed that more than one plasma protein fraction had caused increases in ESR. The complexity of factors affecting the erythrocyte sedimentation rate was confirmed.

SUMMARY

It is suggested from this study that the determination of erythrocyte sedimentation rate is a useful diagnostic aid to clinical medicine. The test appears to be of more use in the evaluation of clinical illness of the dog. Erratic results seen in examination of cat blood limits its usefulness. Two methods of determining ESR in dogs and cats are compared.* The influence of various plasma factors on ESR are reported and discussed. This report indicates that the primary factors influencing ESR are located in the plasma protein components of the blood. Specific identification of the component responsible for causing increases in ESR was not accomplished, although the data strongly incriminates plasma fibrinogen. It is concluded that there are many factors which can influence erythrocyte sedimentation and it is difficult to determine the relative significance of each.

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*The rate of erythrocyte sedimentation is measurable with more confidence in the Wintrobe sedimentation tube than in the Landau-Adams microsedimentation pipette.

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APPENDIX

Appendix Table 1. Anamesis and Results of Leukocyte Studies on Normal Dogs One to Two Months Old (Group I)

Animal Hospital Number	Clinical Diagnosis	Age	Sex	Breed	Total WBC x 10^3	Percentage Distribution* Bas.	Eos.	Lym.	Mon.	Neut.	Band.	Absolute Distribution* x 10^3 Bas.	Eos.	Lym.	Mon.	Neut.	Neut. B.
66-10874	Normal	2 mo.	F	Old Eng. Sheep	14.7	0	11	37	7	42	0	0	1.6	5.4	1.0	6.2	0
66-11582	Normal	1 mo.	M	St. Bern. Mix	11.7	0	4	36	2	58	0	0	0.5	4.2	0.2	6.8	0
66-11583	Normal	1 mo.	M	St. Bern. Mix	12.8	0	2	32	2	64	0	0	0.3	4.1	0.3	8.2	0
66-11584	Normal	1 mo.	F	St. Bern. Mix	10.8	0	1	48	0	51	0	0	0.1	5.2	0	5.5	0
66-11585	Normal	1 mo.	F	St. Bern. Mix	9.7	0	1	53	5	41	0	0	0.1	5.2	0.5	4.0	0
66-11586	Normal	1 mo.	F	St. Bern. Mix	10.8	0	0	40	3	57	0	0	0	4.3	0.3	6.2	0
66-11587	Normal	1 mo.	F	St. Bern. Mix	11.0	0	0	56	2	42	0	0	0	6.2	0.2	4.6	0
66-11588	Normal	1 mo.	M	St. Bern. Mix	13.4	0	1	50	4	45	0	0	0.1	6.7	0.5	6.0	0
66-11589	Normal	1 mo.	M	St. Bern. Mix	11.4	0	4	47	3	46	0	0	0.5	5.4	0.3	5.2	0
66-14140	Normal	2 mo.	F	Eng. Setter	14.4	0	1	55	7	37	0	0	0.1	7.9	1.0	5.3	0
Mean					12.1												

*abreviations reported in differential examinations
Bas. = Basophiles
Eos. = Eosinophils
Lym. = Lymphocytes
Mon. = Monocytes
Neut.= Neutrophils
Band.= Stab neutrophils

Appendix Table 2. Anamesis and Results of Leukocyte Studies on Normal Dogs Six Months to One Year Old (Group II)

Animal Hospital Number	Clinical Diagnosis	Age	Sex	Breed	Total WBC x 10^3	Percentage Distribution* Bas.	Eos.	Lym.	Mon.	Neut.	Band.	Absolute Distribution* x 10^3 Bas.	Eos.	Lym.	Mon.	Neut.	Neut. B.
66- 8799	Normal	7 mo.	M	Mix	19.8	0	6	39	2	53	0	0	1.2	7.7	0.4	10.5	0
66- 8848	Normal	6 mo.	M	Wirehair Terr.	12.4	0	9	39	0	52	0	0	1.1	4.8	0	6.4	0
66-10844	Normal	8 mo.	F	Germ. Shep.	11.2	0	11	25	4	60	0	0	1.2	2.8	0.4	6.7	0
66-11269	Normal	9 mo.	F	Brittary Sp.	18.3	0	4	25	2	68	0	0	0.7	4.6	0.4	12.5	0
66-12207	Normal	6 mo.	M	Brittary Sp.	17.1	0	14	24	5	57	0	0	2.4	4.1	0.9	9.7	0
66-12557	Normal	9 mo.	M	Mix Terrier	12.8	0	7	14	2	75	0	0	0.9	1.8	0.3	9.6	0
66-12780	Normal	12 mo.	M	Mix	10.7	0	1	14	2	83	0	0	0.1	1.5	0.2	8.9	0
66-13145	Normal	7 mo.	M	Doberman	6.2	0	7	24	2	64	0	0	0.4	1.5	0.1	4.0	0
66-13410	Normal	12 mo.	F	Mix	10.8	0	12	14	1	73	0	0	1.3	1.5	0.1	7.9	0
66-13411	Normal	6 mo.	M	Mix	8.3	0	6	34	3	57	0	0	0.5	2.8	0.2	4.7	0
66-13418	Normal	10 mo.	M	Germ. Shep.	13.0	0	3	18	2	77	0	0	0.4	2.3	0.3	10.0	0
66-14151	Normal	6 mo.	M	Germ. Shep.	11.2	0	17	27	6	50	0	0	1.9	3.0	0.7	5.6	0
Mean					12.6												

*abreviations reported in differential examinations
Bas. = Basophiles
Eos. = Eosinophils
Lym. = Lymphocytes
Mon. = Monocytes
Neut.= Neutrophils
Band.= Stab neutrophils

Appendix Table 3. Anamnesis and Results of Leukocyte Studies on Normal Dogs Two to Six Years Old (Group III)

Animal Hospital Number	Clinical Diagnosis	Age	Sex	Breed	Breed WBC x 10^3	Percentage Distribution* Bas.	Eos.	Lym.	Mon.	Neut.	Band.	Absolute Distribution* x 10^3 Bas.	Eos.	Lym.	Mon.	Neut.	Neut. B.
66- 8628	Normal	2 yr.	M	Walker Hound	15.2	0	12	20	2	66	0	0	1.8	3.0	0.3	10.0	0
66-11265	Normal	4 yr.	M	Brittany Sp.	13.8	0	27	24	2	47	0	0	3.7	3.3	0.3	6.5	0
66-11266	Normal	4 yr.	M	Brittany Sp.	15.0	0	6	31	6	56	0	0	0.9	4.6	0.9	8.4	0
66-11267	Normal	3 yr.	M	Brittany Sp.	16.8	0	21	17	3	51	0	0	3.5	2.9	0.5	8.6	0
66-11268	Normal	6 yr.	F	Brittany Sp.	13.5	0	1	24	1	72	0	0	0.1	3.2	0.4	9.7	0
66-12208	Normal	3 yr.	F	Brittany Sp.	17.2	0	12	23	2	63	0	0	2.1	4.0	0.3	10.8	0
66-12209	Normal	4 yr.	F	Brittany Sp.	12.9	0	11	22	5	62	0	0	1.4	2.8	0.6	8.0	0
66-12758	Normal	6 yr.	M	Beagle	11.9	0	16	23	2	59	0	0	1.9	2.7	0.2	7.0	0
66-13580	Normal	2 yr.	F	Germ. S. H. pointer	19.4	0	16	20	2	62	0	0	3.1	3.9	0.4	12.0	0
66-13403	Normal	4 yr.	F	Eng. pointer	7.3	0	8	36	3	53	0	0	0.6	2.6	0.2	3.9	0
66-14175	Normal	5 yr.	F	Germ. Shep.	16.9	0	10	25	3	62	0	0	1.7	4.2	0.5	10.5	0
Mean					14.5												

*abreviations reported in differential examinations
Bas. = Basophiles
Eos. = Eosinophils
Lym. = Lymphocytes
Mon. = Monocytes
Neut. = Neutrophils
Band. = Stab neutrophils

Appendix Table 4. Anamesis and Results of Leukocyte Studies on Normal Cats Two to Three Months Old (Group I)

Animal Hospital Number	Clinical Diagnosis	Age	Sex	Breed	Total WBC x 10^3	Percentage Distribution*						Absolute Distribution* x 10^3					
						Bas.	Eos.	Lym.	Mon.	Neut.	Band.	Bas.	Eos.	Lym.	Mon.	Neut.	Neut. B.
Cat 3	Normal	3 mo.	M	Dom. S. H.	18.5	0	2	42	0	56	0	0	0.4	7.8	0	10.4	0
Cat 4	Normal	3 mo.	M	Dom. S. H.	18.7	0	6	38	1	56	0	0	1.1	7.1	0.2	10.5	0
Cat 5	Normal	10 wk.	F	Dom. S. H.	14.5	0	0	30	0	69	0	0	0.1	4.4	0	10.0	0
Mean					17.2												

*abreviations reported in differential examinations

Bas. = Basophiles

Eos. = Eosinophils

Lym. = Lymphocytes

Mon. = Monocytes

Neut. = Neutrophils

Band. = Stab neutrophils

Appendix Table 5. Anamnesis and Results of Leukocyte Studies on Normal Cats Six to Fifteen Months Old (Group II)

Animal Hospital Number	Clinical Diagnosis	Age	Sex	Breed	Total WBC x 10^3	Percentage Distribution* Bas.	Eos.	Lym.	Mon.	Neut.	Band.	Absolute Distribution* x 10^3 Bas.	Eos.	Lym.	Mon.	Neut.	Neut. B.
Sam	Normal	7 mo.	M	Persian	16.5	0	3	72	0	25	0	0	0.5	11.9	0	4.1	0
Bacar	Normal	8 mo.	M	Siamese	16.0	0	1	52	0	47	0	0	0.2	8.3	0	7.5	0
Rapunsel	Normal	7 mo.	F	Persian	12.6	0	10	53	1	36	0	0	1.3	6.7	0.1	4.5	0
Brand X	Normal	8 mo.	F	Siamese	9.0	0	2	55	4	39	0	0	0.2	5.0	0.4	3.5	0
Roscoe	Normal	7 mo.	M	Persian	15.0	0	7	77	0	16	0	0	1.1	11.6	0	2.4	0
Cat 6	Normal	10 mo.	M	Dom. S. H.	12.1	0	6	20	2	72	0	0	0.7	2.4	0.2	8.7	0
Cat 7	Normal	10 mo.	M	Dom. S. H.	15.2	0	10	36	3	51	0	0	1.5	5.5	0.5	7.6	0
Cat 9	Normal	7 mo.	F	Dom. S. H.	14.3	0	2	52	2	44	0	0	0.3	7.4	0.3	6.3	0
Cat 10	Normal	6 mo.	F	Dom. S. H.	17.5	0	6	29	4	61	0	0	1.1	5.1	0.7	10.7	0
Cat 11	Normal	7 mo.	M	Dom. S. H.	11.8	0	6	47	4	43	0	0	0.7	5.5	0.5	5.1	0
Cat 12	Normal	7 mo.	F	Dom. S. H.	15.4	0	5	38	1	54	2	0	0.8	5.9	0.2	8.3	0.3
Cat 14	Normal	7 mo.	M	Dom. S. H.	14.6	0	5	26	3	66	0	0	0.7	3.8	0.4	9.6	0

Appendix Table 5. (continued

Animal Hospital Number	Clinical Diagnosis	Age	Sex	Breed	Total WBC x 10^3	Percentage Distribution* Bas.	Eos.	Lym.	Mon.	Neut.	Band.	Absolute Distribution* x 10^3 Bas.	Eos.	Lym.	Mon.	Neut.	Neut. B.
Cat 15	Normal	15 mo.	M	Dom. S. H.	15.7	0	1	48	1	50	0	0	0.2	7.5	0.2	7.9	0
Cat 17	Normal	15 mo.	F	Dom. S. H.	18.2	0	4	9	2	85	0	0	0.7	1.6	0.4	15.5	0
Mean					14.6												

*abreviations reported in differential examinations

Bas. = Basophiles

Eos. = Eosinophils

Lym. = Lymphocytes

Mon. = Monocytes

Neut. = Neutrophils

Band. = Stab neutrophils

Appendix Table 6. Anamesis and Results of Leukocyte Studies on Normal Cats Two to Six Years Old (Group III)

Animal Hospital Number	Clinical Diagnosis	Age	Sex	Breed	Total WBC x 10^3	Percentage Distribution* Bas.	Eos.	Lym.	Mon.	Neut.	Band.	Absolute Distribution* x 10^3 Bas.	Eos.	Lym.	Mon.	Neut.	Neut. B.
66- 8072	Normal	5 yr.	M	Siamese	11.6	0	6	55	1	38	0	0	0.7	6.4	0.1	4.4	0
66-10637	Normal	3 yr.	F	Dom. S. H.	16.6	0	19	53	1	25	0	0	3.2	8.8	0.2	4.5	0
Mary Ann	Normal	2 yr.	F	Dom. S. H.	7.6	0	7	44	1	48	0	0	0.5	3.3	0.1	3.6	0
Peggy	Normal	2 yr.	F	Dom. S. H.	17.6	0	5	37	2	56	0	0	0.9	6.5	0.4	9.9	0
Spook	Normal	2 yr.	F	Siamese	9.5	0	3	47	1	49	0	0	0.3	4.5	0.1	4.7	0
Cat 1	Normal	2 yr.	F	Dom. S. H.	17.0	0	0	28	0	71	0	0	0	4.8	0	12.1	0
Cat 2	Normal	6 yr.	F	Dom. S. H.	18.5	0	5	31	0	64	0	0	0.9	5.7	0	11.8	0
Cat 8	Normal	2 yr.	M	Dom. S. H.	17.3	0	10	50	3	37	0	0	1.7	8.7	0.5	6.4	0
Cat 13	Normal	2 yr.	F	Dom. S. H.	7.8	0	7	21	2	70	0	0	0.5	1.6	0.2	5.5	0
Cat 16	Normal	2 yr.	F	Dom. S. H.	18.6	0	1	23	1	75	0	0	0.2	4.3	0.2	14.0	0
Mean					14.2												

*abreviations reported in differential examinations
Bas. = Basophiles
Eos. = Eosinophils
Lym. = Lymphocytes
Mon. = Monocytes
Neut. = Neutrophils
Band. = Stab neutrophils

Appendix Table 7. Anamnesis and Results of Leukocyte Studies on Diseased Dogs Four Months to One Year Old (Group I)

Animal Hospital Number	Clinical Diagnosis	Age	Sex	Breed	Total WBC x 10^3	Percentage Distribution*						Absolute Distribution x 10^3*					
						Bas.	Eos.	Lym.	Mon.	Neut.	Band.	Bas.	Eos.	Lym.	Mon.	Neut.	Neut. B.
66-13582	Subacute canine distemper	7 mo.	M	Mix	12.2	0	17	37	0	46	0	0	2.1	4.5	0	5.6	0
66-13904	Acute spondylitis	1 yr.	M	Dachsand	10.5	0	0	9	6	85	0	0	0	0.9	0.6	8.9	0
66-13907	Chronic distemper	4 mo.	F	Beagle	12.6	0	0	4	0	94	2	0	0	0.5	0	11.8	0.3
66-13911	Acute distemper	1 yr.	M	Spitz	7.1	0	10	22	3	85	0	0	0.7	1.6	0.2	8.9	0

*abreviations reported in differential examinations

Bas. = Basophiles

Eos. = Eosinophils

Lym. = Lymphocytes

Mon. = Monocytes

Neut. = Neutrophils

Band. = Stab neutrophils

Appendix Table 8. Anamnesis and Results of Leukocyte Studies on Diseased Dogs Two to Seven Years Old (Group II)

Animal Hospital Number	Clinical Diagnosis	Age	Sex	Breed	Total WBC x 10^3	Percentage Distribution						Absolute Distribution* x 10^3					
						Bas.	Eos.	Lym.	Mon.	Neut.	Band.	Bas.	Eos.	Lym.	Mon.	Neut.	Neut. B.
Blaugh D	Chronic distemper	6 yr.	M	Scottie	9.9	0	10	27	0	63	0	0	1.0	2.7	0	6.2	0
66-13723	Subacute distemper	2 yr.	M	Old Eng. Sheep	7.8	0	0	11	0	89	0	0	0	0.9	0	6.9	0
66-13821	Chronic pneumonia	4 yr.	M	Brittany Sp.	25.1	0	23	16	0	61	0	0	5.8	4.0	0	15.3	0
66-13906	Endometritis	3 yr.	F	Beagle	11.9	0	23	20	3	54	0	0	2.7	2.4	0.4	6.5	0
66-14314	Acute Enteritis	7 yr.	F	Poodle	18.8	0	2	8	8	82	0	0	0.4	1.5	1.5	15.4	0
66-14321	Acute Nephritis	3 yr.	M	Terrier Mix	16.2	0	1	27	0	72	0	0	0.2	4.4	0	11.7	0
66-14325	Metritis	5 yr.	F	Eng. Pointer	12.1	0	18	11	0	71	0	0	2.2	1.3	0	8.6	0
66-14342	Osteomyelitis	6 yr.	M	Great Dane	18.0	0	5	14	0	81	0	0	0.9	2.5	0	14.6	0

Appendix Table 8. (continued)

Animal Hospital Number	Clinical Diagnosis	Age	Sex	Breed	Total WBC x 10^3	Percentage Distribution* Bas.	Eos.	Lym.	Mon.	Neut.	Band.	Absolute Distribution* x 10^3 Bas.	Eos.	Lym.	Mon.	Neut.	Neut. B.
66-14414	Acute hepatitis	5 yr.	F	Terrier Mix	18.4	0	2	16	0	82	0	0	0.4	3.0	0	15.1	0
66-14836	Subacute distemper	3 yr.	F	Brittany Sp.	10.2	0	3	5	3	89	0	0	0.3	0.5	0.3	9.1	0

*abreviations reported in differential examinations

Bas. = Basophiles

Eos. = Eosinophils

Lym. = Lymphocytes

Mon. = Monocytes

Neut. = Neutrophils

Band. = Stab neutrophils

Appendix Table 9. Anamesis and Results of Leukocyte Studies on Diseased Dogs Eight to Eleven Years Old (Group III)

Animal Hospital Number	Clinical Diagnosis	Age	Sex	Breed	Total WBC x 10^3	Percentage Distribution* Bas.	Eos.	Lym.	Mon.	Neut.	Band.	Absolute Distribution* x 10^3 Bas.	Eos.	Lym.	Mon.	Neut.	Neut. B.
66-10454	Interdig. cysts	9 yr.	M	Eng. Pointer	13.3	0	7	16	3	80	0	0	0.9	2.1	0.4	10.6	0
66-13910	Toxic hepatitis	8 yr.	F	Bassett	17.0	0	1	13	7	73	0	0	0.2	2.2	1.2	12.4	0
66-14514	Staph. Dermatitis	11 yr.	F	Germ. Shep.	18.4	0	10	12	0	78	0	0	1.8	2.2	0	14.3	0

*abreviations reported in differential examinations

Bas. = Basophiles

Eos. = Eosinophils

Lym. = Lymphocytes

Mon. = Monocytes

Neut. = Neutrophils

Band. = Stab neutrophils

THE INFLUENCE OF SELECTED FACTORS ON ERYTHROCYTE SEDIMENTATION RATE IN THE DOG AND CAT

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Use of the erythrocyte sedimentation rate (ESR) as a non-specific laboratory examination in evaluating a clinical patient has increased over the past half century. Knowledge of the factors which influence the erythrocyte sedimentation rate has accumulated slowly.

Review of previously published reports indicated that of the erythrocytic, physical, and plasma factors which were known to influence ESR, the plasma factors were of greatest clinical significance if technical variables were limited. Plasma fibrinogen elevations were reported to be of primary significance in increasing erythrocyte sedimentation rates. The non-specificity of the ESR phenomenon was established by experimental evaluations that suggested that any asymmetric molecule of high molecular weight caused increases in erythrocyte sedimentation rates. Little investigation has been conducted on the comparison of erythrocyte sedimentation rates of normal and diseased dogs. Less information was available for evaluation of the ESR as a routinely employed diagnostic aid in feline medicine.

In this study attempts were made to evaluate the influence of cellular constituents, total plasma cholesterol, and plasma protein distribution on ESR in the dog and cat. Erythrocyte sedimentation rates were determined in the Wintrobe sedimentation tube and the Landau-Adams microsedimentation pipette and the data were compared. Total plasma proteins were obtained by methods utilizing differences in specific gravity and refractive index. Relative and absolute distributions of plasma proteins were determined electrophoretically on both normal and diseased dogs and on normal cats using cellulose polyacetate as the supporting medium.

Comparison of ESR values observed in the Wintrobe tube and the Landau-

Adams microsedimentation pipette indicated the questionable values of the latter apparatus. The results obtained employing the microsedimentation pipette were irregular and often failed to indicate the increased extent of erythrocyte sedimentation. The microsedimentation pipette was not recommended for routine use.

Plasma cholesterol values were not related to alterations in ESR.

Plasma protein values obtained by the use of the Banco Density Gradient and Serum Protometer compared favorably in all animals. The results indicated that the albumin-globulin ratio was a more consistent indicator of the duration of illnesses than leukocyte examinations and ESR.

The electrophoretic separation utilizing cellulose polyacetate was felt to be an adequate technique of analysis as fairly consistent values were observed within groups of normal dogs and cats. Comparison of protein distribution changes between normal and diseased dogs suggested that fibrinogen values were practically always elevated when increased ESR results were obtained. Alterations in distributions of other proteins were not consistently associated with increases in ESR. However, alpha globulins were often elevated when ESR was accelerated. There was no apparent correlation between changes found in albumin, beta and gamma globulin fractions with ESR. These findings were in agreement with investigations reported in the literature.

It was concluded from this study that alterations in erythrocyte sedimentation were not related consistently to other blood changes and that this phenomenon appeared to be dependent on a number of interacting factors in the plasma.